RECOMMENDATIONS ON AQUATIC BIOLOGICAL TESTS AND PROCEDURES FOR ENVIRONMENTAL PROTECTION, C & P, DOE

CONSERVATION & PROTECTION
ENVIRONMENT CANADA

PREFACE

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Comments concerning the report should be directed to either the Chairman of LMC, or the author, G.A. Sergy.

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RECOMMENDATIONS
ON AQUATIC BIOLOGICAL TESTS AND PROCEDURES
FOR ENVIRONMENTAL PROTECTION, C & P, DOE.

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EXECUTIVE SUMMARY AND RECOMMENDATIONS

Environment Canada recognizes the need for the comprehensive management of toxic chemicals and for a holistic approach in protecting the interacting natural systems of the environment and its human population. To achieve this worthy but difficult goal requires sound and practical tools that can be used to 'predict, prevent and control'. To this end, Conservation and Protection would benefit from the development of a balanced ecotoxicological testing capability and a more effective use of aquatic toxicity tests and biomonitoring procedures.

Report Summary by Chapter

1.0 Purpose and Concerns

This study was initiated because of concerns about the role of biological tests and in-house aquatic toxicity testing capabilities required in Environmental Protection (EP), Conservation and Protection (C&P). There was concern about the inconsistency nationally in the amount, manner and effectiveness of present biological test use. There was concern that EP was not adequately preparing for future operating needs (eg. CEPA) and was not in phase with the increased recognition and use of biological tests by agencies elsewhere (eg: U.S. EPA Water Quality-based Toxics Control, Ontario MISA program, OECD).

Moreover, the situation is not compatable with federal government initiatives to provide a "comprehensiveness to environmental protection", "a total environmental quality strategy", and "leadership in the establishment of nationally consistent standards".

In addressing the above concerns this report provides recommendations for management decisions and action to strengthen the usage and improve the availability of aquatic biological tests and procedures in environmental protection activities. The report provides guidance in putting basic acceptable tools on-line to satisfy EP needs and responsibilities. Emphasis is placed on greater standardization to improve quality and efficiency. The study addresses the subject primarily from a national perspective.

2.0 Advantages and Limitations of Biological Testing

A short discussion is provided on what is already widely accepted on biological testing: when used in a judicious manner it can improve the practice of environmental protection; it should be used jointly and in the context of other information (e.g. chemical) that forms a logical and integrated approach to problem identification, problem solving and decision-making; and the limitations can be recognized and dealt with acceptably.

3.0 Applications of Biological Testing

A survey of EP managers identified current and potential uses for biological testing in EP activities. Not unexpectedly, potential use far exceeded actual use, and the latter was highly variable across the country. Those same factors which were responsible for this condition, and the concerns which initiated this report, likewise precluded a quantitative projection of test needs. Those factors include; EP's diffuse and variable role and operating practice; the nature of or lack of environmental standards; the lack of methodology, capability and resources.

The purpose for biological testing is defined and discussed in twelve general 'application' categories and under the four major processes of: problem identification; assessment; control; and evaluation. These are consistent with current thinking and collectively encompass the needs of EP and C&P. On the basis of assumed EP national priorities, emphasis is put on those 'applications' for 1) identification and investigation of toxic chemical hazards, 2) development and evaluation of waste treatment technology and disposal methods, 3) development and evaluation of national 'standards' of environmental quality and protection, 4) audit and enforcement of regulatory standards.

Both the letter and spirit of the draft Canadian Environmental Protection Act (CEPA) support the use of biological testing, although the full extent of that use remains to be clarified.

4.0 A Selection of National Core Aquatic Toxicity Tests

A number of individual aquatic toxicity tests are selected to form a national core set. That selection is based on the best fit with various criteria set forth in this chapter. The toxicity tests selected for the national core set include those to measure:

- lethality in rainbow trout, 3-spine stickleback, daphnids, brine shrimp and amphipods (sediment assay)
- sublethal effects in rainbow trout (ATP stress), fathead minnows (growth inhibition), daphnids (reproduction or life cycle impairment), algae (growth inhibition), and bacteria (metabolic)
- mutagenic or genotoxic effects
- bioconcentration and biodegradability.

The core set is broadly applicable to a wide range of environmental protection activities. It satisfies existing and proposed national legislation and perceived national role/priorities. Most of the core tests are recognized and accepted by other agencies, which has led to the development of a considerable knowledge base. The selection is consistent with OECD guidelines and U.S. EPA regulatory use. Collectively, the core set takes into account acute and chronic toxicity and different species and trophic levels from freshwater and marine environments. Insofar as possible, the selection is made of tests that are practical and have relevant end points.

Provision is made to identify toxicity tests for specialized regional or program needs such as would be required and justified on a case-by-case/responsibility centre basis. Field biomonitoring procedures are described only in general terms. It is concluded that definitions of national freshwater field biomonitoring procedures/programs would best be done jointly by EP and IWL (Inland Waters and Lands) and following clarification of biomonitoring issues and objectives.

5.0 A Toxicity Testing Capability

It is concluded that C&P must maintain a strong technical and scientific knowledge base and expertise, and in-house testing capability. Such a policy is consistent with public opinion, Departmental responsibilities and mandate and all recent initiatives related to toxic chemicals (Environmental Protection Act, cradle-to-grave management). The present and potential value and importance of in-house capabilities are considered to be large when compared to the resource requirements.

Recommendations for a national core aquatic toxicity testing capability are provided, including the distribution of tests geographically, the provision of service and the implementation of additional capabilities in a phased approach over the next two years.

6.0 Core Test Cost Estimates

Where possible, estimates are provided on resources to implement a national core aquatic toxicity testing capability. Overall, it is felt that total resource requirements are not prohibitive; they are largely PY-related; they are within the means of C&P. Cost-per-test data estimates are provided for government labs and the private sector.

7.0 National Protocol and Procedure Development

A strategy is proposed to develop protocols and procedures for the national core aquatic toxicity tests and species selected earlier. Provision is made for a fast-track or traditional track approach to drafting the protocols and for a wide review prior to publication as a DOE testing methodology. Resource needs and a schedule for protocol development call for the provision of one PY and $50K O/M per year for a five year period.

8.0 Quality Assurance

Recent developments in QA are discussed including Good Laboratory Practice (GLP). An overview of major quality assurance elements is presented. Concerns are expressed about quality assurance programs within DOE and for contracted services. Recommendations to correct the situation are provided.

9.0 Methodology Research and Development

Major areas for R&D efforts are identified and discussed as are options for getting such work done. It is concluded that some basic in-house level of effort is beneficial and required to conduct practical methods development work.

Recommendations Summary

It is recommended that an information and decision package be prepared for C&P senior management which requests:

1) a policy statement on the use of biological tests and procedures in environmental protection and management, and clarification on that use with respect to attaining desirable environmental quality levels;
2) support for EP initiatives to establish and maintain an in-house capability for the conduct of a balanced set of well defined aquatic toxicity tests (as outlined) and for the development and publication of Canadian protocols and procedures for those tests.
3) coordination of the laboratory activities of EP and Inland Waters and Lands (IWL), with respect to the provision of in-house toxicity testing services and methods development, as required to ensure the efficient use of existing resources.

It is recommended that the Lab Managers Committee (LMC)

1) approve the suggested content and provide a decision package to senior management (as outlined above);
2) adopt the national core aquatic toxicity tests as defined for a national in-house testing capability;
3) implement the recommendations and guidance provided on the distribution of toxicity tests, provision of service and a quality assurance program;
4) support and coordinate systematic efforts for the development and adoption of national protocols and procedures for all core toxicity tests;

Implications

Recommendations and guidance provided in this report are not administratively or financially unreasonable. Likewise they are not rigid and should be kept flexible in order to reflect changing conditions.

Corrective actions with respect to biotesting are however considered imperative for the integrity of C&P and of the Canadian environment. A progressive stance must be taken to address both current problems and the anticipated needs. Failure to do so is predicted to result in future public criticism, in damaged scientific credibility and the inability to deliver the best level of environmental protection to the people of Canada.

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1.0 INTRODUCTION

1.1 Purpose and Scope of this Study

Many organizations, agencies, and individual practitioners are now advocating the wider use of biological indices of environmental quality and the use of toxicity tests in combination with chemical and microbiological analyses in order to predict and control environmental deterioration. However, concerns have been voiced: over the uncertainty in EP* with respect to the role of biological tests and the capability EP should have to perform such work; over the variation nationally in the amount, manner and effectiveness of biological test use; that EP as a whole is not adequately preparing for future operating needs in line with increased recognition given elsewhere. In support of the efforts of many others on this problem, the present study was commissioned by the EP Lab Managers Committee (LMC) with the hope of influencing both the future availability and usage of biological testing options.

The primary goal of this study is to see that the right kinds of biological tests are placed at the disposal of EP managers; to identify a balanced set of applicable aquatic toxicity tests and provide guidance in putting basic quality tools 'on-line'. The second purpose is to further promote program managers' growing acceptance and understanding of biological tests and their use in an interdisciplinary approach to environmental protection.

The report deals with the subject primarily from a national perspective. It discusses present and potential applications of biological tests, selects a 'best fit' set of aquatic toxicity tests, and addresses the provision of those tests in EP and in-house capability. Emphasis is placed on greater standardization to improve the quality and usefulness of the product.

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* refers to Environmental Protection, Conservation and Protection, previously known as EPS, Environmental Protection Service.

This report deals with biological tests and procedures for the protection of water quality and aquatic life primarily from the consequences of toxic pollution. Attention is focused on toxicity tests as opposed to conventional field biomonitoring procedures, recognizing, however, that both are required for a truly holistic approach to environmental protection and management of our aquatic resources. The report considers: both freshwater and marine environments; biological tests or procedures applied in the lab and field; complex effluents and leachates and single chemical sources; and the prediction and measurement of toxic effects, either before or after release to the environment.

The report primarily deals with those matters which are within the area of responsibility of EP. However, wherever possible the Conservation and Protection (C&P) perspective is adopted. Responsibility centres are often considered on an individual basis because of their dissimilarities. For EP they are defined as: Pacific and Yukon Region (P&Y); Western and Northern Region (W&N); Ontario Region (Ont); Quebec Region (Que); Atlantic Region (Atl); Industrial Programs Branch (IPB); Commercial Chemicals Branch (CCB); Technology Development and Technical Services Branch (TDSB); and the Management and Emergencies Branch (MEB).

It should be recognized that this report must be considered in the broader context of (1) departmental activities, (2) assessments of ecotoxicity in aquatic, terrestrial and aerial components of the biosphere and (3) the measurement of ecotoxicity by other available techniques.

What is Ecotoxicity?

The study of ecotoxicity is the study of "toxic effects of physical and chemical agents on living organisms, especially on populations and communities with defined ecosystems; it includes the transfer pathways of those agents and their interactions with the environment" (Butler 1978).

An ecotoxicological approach to environmental protection is an ecologically relevant assessment of problems utilizing biological and chemical procedures in a complementary fashion. Both exposure and effects must be considered at various ecosystem levels.

Such an approach is required to most effectively deal with the threat of toxic substances and maintain environmental quality i.e. "that condition of the environment necessary for the health and well being of people, the health and diversity of species and of ecosystems, and the sustained use of natural resources"...

1.2 Policies of Other Agencies on Ecotoxicity and The Use of Biological Tests

United States Environmental Protection Agency

In the U.S. it is national policy that the discharge of toxic pollutants in toxic amounts be prohibited. It is also their goal that zero discharge of pollutants to waters be achieved. Because this is not immediately attainable, provision is made to issue National Pollutant Discharge Elimination System (NPDES) permits for wastewater discharges. The permits contain technology-based effluent limits and where these do not protect water quality, then water quality-based limits are included. Those limits are influenced by water quality standards. The standards designate a use category, include narrative and numerical criteria specific to the designated use and contain an antidegradation clause. Numerical standards can be chemical-specific (i.e. concentrations) or whole-effluent specific (i.e. acute toxicity). Narrative standards include a criterion which specifies that waters shall be free from "substances in concentrations or combinations toxic to or producing adverse physiological responses in humans, wildlife or aquatic life". This standard is used as a basis to limit both individual toxicants and whole effluent toxicity. To assist in the interpretation and application of this standard the U.S. EPA issued a new national policy in 1984; "Development of Water Quality-Based Permit Limitations for Toxic Pollutants" (Appendix 1A). The policy supports an integrated strategy consisting of both conventional and biological methods to address toxic and non-conventional pollutants, i.e. the use of both chemical and biological approaches (see Appendix 1B for a comparison). Several EPA guidance documents have been written as a result of this policy (EPA 1985a) (EPA 1987).

Rationale for Using Biomonitoring in Permitting

Regulators in the U.S. encountered major problems in the control of toxics, particularly in complex effluents. For example:

- the vast number and diversity of toxic substances that may be potentially discharged
- the difficulty of detecting and monitoring toxics which were frequently unknown and the associated high costs of chemical characterization
- the lack of basic toxicology data for many substances and the limited effluent toxicity data base
- the inability to predict the effect of exposure to combinations of substances and to account for changes in effects resulting from reactions within the matrix of constituents
- the potential long term effects of toxics.

To address these types of problems EPA has put recent emphasis on whole-effluent toxicity control - on the increased use of aquatic toxicity tests to complement chemical analysis and the chemical-specific approach to effluent control. In other words, they intend to determine and use overall toxicity directly without necessarily identifying and analyzing each contributing toxic substance.

This complementary approach is enhanced by research which has convinced EPA that

- the variability inherent in toxicity testing can be controlled to acceptable levels (EPA 1985a)
- adequate correlation exists between an impact predicted by the toxicity tests and an actual effect detected by effects surveys (Mount et al. 1984).

EPA has published procedural manuals for the measurement of acute toxicity (EPA 1985b) and short term chronic toxicity (EPA 1985c) of effluents.

Ontario Ministry of the Environment

Ontario MOE is currently reassessing strategies for effluent control in a major program called MISA (Municipal and Industrial Strategy for Abatement). Although the emphasis is on toxics, MISA will control conventional pollutants as well. The program is to be initially implemented through the development of two regulations per sector. The first regulation will require dischargers to self-monitor their effluents and report the results. The self-monitoring program will be audited by the Ministry of the Environment. The second regulation will set effluent requirements for each sector which will be based on Best Available Technology Economically Achievable (BATEA). Receiving water bodies will be analyzed to determine if these technology-based effluent limits protect local water quality. If not, more stringent water quality impact-based effluent requirements will be imposed. This will be done on a site-specific basis and through the use of Control Orders. Review of BATEA limits will be repeated over appropriate periods of time and, where necessary, the limits will be tightened as technology improves.

Biomonitoring in MISA: Critical assessments are being made on the use of biological tests. Removal of acute lethal toxicity via BATEA is considered the first step. The 96 hr rainbow trout test and the Daphnia lethality test are the most likely regulatory tests. Other biomonitoring tools which measure chronic, genotoxic and sublethal effects are under evaluation at MISA pilot site studies. These would play a role in the definition of water quality-based controls. The stated position of the MISA working group 4 regarding biomonitoring for all sectors is that "As a minimum, industrial and municipal discharges must be non-acutely lethal to fish. Since the impact of toxic discharges on aquatic organisms ranges from acute lethality through sublethal toxicity leading to adverse chronic effects, further appropriate effluent biomonitoring tests should be applied (and/or developed) on an industry or sector-specific basis."

Other Organizations

Long active in the development of Chemical Guidelines, the Organization for Economic Co-Operation and Development has recently concluded "that toxicity testing procedures are founded on a sound and generally accepted scientific basis. Evaluation of effluent toxicity, particularly when analysed in conjunction with chemical and ecological data, can provide a valid indication of the effects of toxic effluents on receiving systems, and can significantly improve the development of regulatory requirements to protect aquatic life. Such toxicity testing procedures are a tool which should be used in OECD countries (in conjunction with other appropriate techniques) to identify, quantify and control the discharge of toxic pollutants by industrial facilities and municipal sewage treatment plants" (OECD 1986).

The United Nations Environment Program (UNEP) 1985 guidelines also build biological procedures into a fundamental environmental protection framework for use in evaluating and controlling pollutants entering estuarine and coastal waters, as do agencies such as the International Council for Exploration of the Sea (ICES), Intergovernmental Oceanographic Commission (IOC), and the Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP), (see Wells et al. 1986). There is general endorsement of the role and value of biological procedures in environmental protection.

2.0 ADVANTAGES AND PROBLEMS WITH THE USE OF BIOLOGICAL TESTS

Environmental Managers must be able to

- identify problems at an early stage
- assess the need for and define the nature of control action
- control use and release of toxic substances
- assess the effectiveness of controls.

Biological tests and procedures can aid decision makers in these tasks by predicting and detecting effects, screening and prioritizing problems, and by quantitatively estimating hazards. They can be used to set and enforce standards, and to evaluate corrective actions. In doing so they support the cognitive, preventative and remedial functions essential to EP performance. They may be used in a one-time "single-shot" mode or in a monitoring capacity. They include measurements of toxicity using captive organisms exposed in controlled conditions and the measurement of toxic substance distribution and effects in the receiving environment.

2.1 Benefits

Biological tests and procedures, when used in a judicious and effective manner, can improve the quality of environmental protection.

1) Biological tests may provide critical information relevant to decision-making. The quality of decisions can be improved with a greater knowledge base. Likewise, there may be more confidence in judgements rendered with evidence of cause-effect relationships.

2) Biological tests can provide less costly alternatives in some situations as well as enhance the efficiency and effectiveness of physical-chemical analyses. Biological tests tend to be cheaper than complex organic analyses. With the trend toward the latter, and resource restraints, biotesting options are a practical component of analytical programs.

3) Biological tests provide perceptible results which may be more credible, convincing or understandable in illustrating environmental issues to the public and industry. They may be used to inflate or defuse issues.

4) Biological tests are directly correlated with the potential effects of pollutants. They can thus permit the protection of the environment effectively by allowing the application of the right level of pollution control compatible with the desired water quality objectives.

5) Biological tests allow assessment of the long-term effects of toxics and some insight into potential future consequences of our actions.

6) Toxicity as measured by biological tests can be regulated as a generic pollutant and can overcome a large number of problems inherent in other approaches to toxics control. The U.S. EPA has recognised the need for whole effluent toxicity control (see Chapter 1.2). Their experiences have shown that the time, costs and ability to identify all constituents of a complex effluent can be prohibitive. A complex waste may have hundreds of substances and it is extremely difficult and expensive to determine which are the problem. Furthermore, toxicity data or water quality criteria do not exist for many chemicals. Biological tests also provide a solution to deal with interactions which alter toxicity, i.e. physiological effects of multiple toxicants on organisms and matrix effects caused by effluents and receiving waters.

Biology and Chemistry Working Together

It must be emphasized that biological tests and procedures are an ADDITION TO and NOT a REPLACEMENT FOR other control measurements, such as single chemical criteria or other conventional methods.

The use of biological and chemical methods together is essential for producing meaningful results. Blaise et al. (1985) outline the integrated ecotoxicological approach used in the Quebec region and the associated cost/benefit ratio of their hazard assessment scheme (Appendix 2A). They also show how biotests can be useful in relating observed environmental effects to a chemical cause (Appendix 2B). Such capability is expected to improve in the future with the development of bioengineered microorganisms that are sensitive to particular classes of chemicals.

2.2 Limitations and Constraints

The most serious limitations in the use of biological tests and procedures are in fact related to problems of incorrect usage. Lesser concerns over cost, acceptability, technical and operational issues also exist.

Effective Use

Proper choice of test(s) and interpretation and use of results are important, but they do not always occur. One reason is the problem of inadequate communication between decision makers, project managers and scientists. This situation is further aggravated if managers do not or cannot precisely define their goals and biologists do not provide practical and understandable advice.

Some key principles for effective use follow:

1) The right test must be chosen for the job. Understanding when to use a particular test is important to solving the problem as well as to the credibility of the testing program. The choice of a test depends on the questions being asked and the level of confidence required in the answer. The purpose and nature of information sought must be clearly stated.

2) Biological test information must be translated and expressed in pragmatic and useful terms for the decision maker. It must be placed and used in the context of the problem at hand.

3) Judgement calls must be made. Hazard assessment is based in part on scientific judgement. The environment is a living and dynamic system for which all answers do not exist. "It is the mark of an instructed mind to rest easy with that level of precision which the decision requires and not try an exactness which is unnecessary for the problem" (Aristotle).

4) The results must be considered in the context of other information that form logical components of a decision making framework. For example, the toxicity of an effluent cannot be used as the sole means of assessing impact or developing controls but should be used together with results from ambient tests, chemical and physical measurements, the nature of receiving waters, economic and social factors.

Cost

Some types of biological test and procedures can be very expensive, as can chemical analysis, but the quality and type of results are also vastly different. Decision makers should be paying for only the quality, quantity and type of information that is essential to their needs. Obviously, monies are wasted when incorrect selections are made. Criteria are needed for the selection of cost-effective types of tests for different levels of decision. Hard cost-comparative documentation is incomplete.

Cost can also be a limiting factor in the absolute sense, no matter what type of test or control methodology is employed. It has been noted in the U.S. that costs for contract laboratories have started to come down as a result of increasing use of biological tests (Hanmer and Newton 1984).

Acceptability to Stakeholders

Opposition to the use of biological tests can be expected from industry, because of a lack of understanding or for financial reasons. Biological tests may identify the need for a greater level of environmental protection than now exists. Regulators themselves often resist changing an existing system.

Technical Issues

There are a number of general technical issues which can be identified as needing additional effort in order to improve the usefulness of biotests. None are of a prohibitive nature. Included are field validation, standardization, methods to measure toxicity persistence, distribution and accumulation of substances, and guidance on toxicity treatability. EPA (1985a) has taken a clear position on those issues of relevance to their permitting system. These issues and others are discussed in Appendix 2C.

Human Health Concerns

Biological tests for 'environmental effects' have limited ability to predict human health effects or human health risk from pollution sources. More health related tests or better correlation to existing human health tests is required.

Operational Restraints and Requirements

Appropriately trained personnel are the most important operational requirement. Obviously, testing facilities (government or private) must also be available. Mechanisms for standardization and intercalibration are needed for quality control.

3.0 APPLICATIONS OF BIOLOGICAL TESTING IN ENVIRONMENTAL PROTECTION ACTIVITIES

3.1 Past Use of Toxicity Testing in EP

Canada has made extensive use in the past of toxicity data for the control of toxic substances. By and large, previous emphasis in EP was related to the control of effluents, while more recently the focus has been on priority chemical problems such as PCBs, pesticides, specific metals, dioxins, and other organics, with work proceeding under the ECA and Toxic Chemicals Management Program of Environment Canada.

The use of biological (toxicological) procedures has evolved from data acquisition on acute toxicities, to physicochemical and biological parameters being regulated and monitored by industrial sectors by the 1970s, to compliance monitoring and hazard assessments being conducted in the 1980s (Blaise *et al*. 1987). For most of the 1970s, the federal strategy in Canada for control of industrial effluents was to first control discharges at source nationally, within major industrial sectors through national regulations and guidelines (under the Fisheries Act), then to monitor the effectiveness of controls and make adjustments based on a plant-by-plant, site-by-site experience. An acute toxicity protocol with fingerling rainbow trout (*Salmo gairdneri*) was developed and applied to effluents. The objective was to improve final effluent quality so that fish could survive in full-strength effluents, or allowable dilutions, for 4 days. This rainbow trout test continues to be very valuable in Canada for screening and regulating effluents. There were also many studies of acute toxicity reduction within treatment systems and of the sublethal toxicity of treated and untreated effluents, particularly from pulp and paper mills and oil refineries. In the late 70s emphasis started to shift to chemical and biological studies of priority chemicals in receiving waters near industrial outfalls and near areas of large scale chemical use. Some sublethal toxicity procedures were developed to be applied to these situations and non-lethal contaminant sources.

The history, purpose and types of toxicity tests conducted by regional EP laboratories is presented in MacGregor and Wells (1984). Some of their data has been updated to March 1986 (Appendices 3A and 3B herein). The information demonstrates that EP toxicity laboratories have the capacity to perform a number of toxicity tests in support of a variety of objectives, programs and intervention activities. Such a capability satisfies responsibilities under several statutes (Fisheries Act, Ocean Dumping Control Act, International Boundary Waters Treaty Act, Environmental Contaminants Act).

MacGregor and Wells concluded that present resources were barely adequate to meet differing regional operational requirements, but inadequate for a variety of national needs related to chemical testing, hazard assessment, quality assurance, etc. That sitution remains today as EP toxicity labs struggle to provide a viable level of service required to meet national needs. Despite the relatively low level of resourcing, those labs are heavily utilized; for example, the Montreal lab runs 200-300 Microtox and algal assays per year and the Vancouver lab ran 375 rainbow trout assays in 1985 and 425 in the first 9 months of 1986.

3.2 Survey of Present and Potential Uses of Biological Tests

A survey of EP project managers and their review of 86/87 work plans identified current and potential uses for biological tests in environmental protection activities. Addendum I summarizes the input received from the managers and demonstrates the diverse nature of the responses. The programs of each EP region and HQ branch were reviewed in this exercise. Two general conditions are evident from the results of that survey. Not unexpectedly:

1) the purpose, amount and type of biological testing by EP (or the potential use) is highly variable across the country;

2) the potential use of biological testing exceeds actual use by EP.

Factors responsible for one or both of the above conditions include:

- the diffuse and changing role of EP
- unclear policy and practice in compliance and enforcement
- lack of specific 'standards' for desirable levels of environmental quality
- presence or absence of a biological test as a regulatory standard
- unfamiliarity with the cost and level of effort required for biological testing options and the meaning of results generated
- the different regional needs and different arrangements developed with OGDs and provincial agencies
- the lack of methodology, test protocols or testing capability
- the lack of resources

These factors made it impractical to prepare a quantitative or definative projection of test needs. The problem is not whether or not there is a need, but being unable to substantiate that need. Considerable toxicological support could be and should be provided to EP managers.

The data gathered in the survey along with other information on the requirements of C&P (see Chapter 5) was used in the definition of general 'applications' for biological testing. An attempt was also made to classify the survey and past use data according to those applications (Appendix 3C, D, E) however, the most comprehendable depiction of uses and needs can be found in the narrative account of applications presented in Chapter 3.3 below.

3.3 The Perceived Role and Application of Biological Testing in EP and C&P

The purpose for biological tests and procedures was defined in twelve general 'applications' within the four major steps of problem identification, problem assessment, control and control evaluation. These steps and most of the applications are familiar to managers and are consistent with current strategies and thinking in C&P.

Collectively these applications define the role and the need for biological testing in EP and C&P from a national perspective. A definition of each follows.

Application/Role of Biotests in Environmental Protection Activities

A. Problem Identification

1. Identify and Screen Potential Hazards
2. Signal Changes in Environmental Quality

B. Problem Assessment

3. Assess Hazards
4. Assess Receiving Environment Effects
5. Set Ambient Environmental Quality Objectives

C. Control or Intervention

6. Evaluate Waste Treatment and Disposal Methods
7. Apply Site Specific Controls
8. Apply a National Level of Protection
9. Enforce Regulatory Standards

D. Control Evaluation

10. Monitor Compliance to Control Measures
11. Monitor Effectiveness of Control Measures
12. Monitor Long Term Environmental Trends

1. Identify and Screen Potential Hazards or Problems in Suspect Samples

Biological testing can be used to identify and characterize the toxicity of a material to various levels of detail depending on the information needs. This could range from a simple qualitative indicator, to a broad spectrum preliminary assessment to a fully quantitative analysis. For ease of discussion, the use is split here into the two categories of problem identification or problem assessment (see 3 below), although in many cases there is a continuum from one to the other.

At the problem identification level toxicity screening data may be used

- to indicate specific concerns
- to predict threats at an early stage
- to provide direction for further analysis and assessment
- to assist in setting priorities

Samples for such testing include: unknown chemical(s) or waste material; chemicals and products where use and release is not controlled; unregulated effluents; airborne contaminants; developmental chemicals or formulations; substances likely to be spilled or those spilled; contaminated sediments or dredge spoils; landfill leachates.

2. Signal Changes in Environmental Quality

Longer-term monitoring for trends (see 12) is an important mechanism to signal changes in environmental quality. Field biomonitoring procedures of a short-term nature can, however, also be used at the problem identification stage to detect changes in the quality of the actual receiving environment in specific situations where environmental degradation is suspected.

A Note on Establishing Priorities for Pollution Control Actions

The setting of priorities is a key element of environmental protection and management. A focus of the very limited resources in EP/C&P (by the most efficient means) is considered essential to future efforts. An objective and systematic use of biotests can assist in this regard. It can allow for example: new or potential problems to be assessed in perspective of the overall workload; selection of the most significant problem/source; development of broad action plans; efficient resources utilization.

Data used to set priorities can be generated by:

- preliminary toxicity screening
- more extensive toxicity assessments
- tiered ecotoxicological evaluations
- contaminant distribution and concentration patterns
- observed degradation of receiving environments.

3. Assess Hazards or Problems in Samples

A more definitive or quantitative characterization of toxicity in samples may be used at the problem assessment stage in order to

- set priorities for action
- define the nature or support the development of control measures.

The same types of substances as those described in 1 (above) might be tested, usually after screening has confirmed them to be of concern. The toxicity data can be used (with other information) for: contingency planning and spill response; evaluating remedial measures for sites where contaminated sediments, soils or water pose a hazard; predicting the assimilative capacity of the receiving environment; and developing site-specific or 'national' standards to control the use or release of toxic substances.

In some cases, the problem assessment may include prediction of effects on target organisms in the receiving environment.

4. Assess Receiving Environment Effects

A documentation of existing environmental degradation may be used

- to demonstrate the need for control measures
- to help define the nature of control measures required.

In some cases only the proof of 'real' impact and effects on resource species will provide the impetus for action by other regulatory agencies/ industry. Clarification of effects may be required for enforcement action. Actual effects information may help clarify sources, causes and significance of toxicity thereby providing guidance in deciding on appropriate controls or remedial action. Such may be the case where problems are large and complicated by technical, economic or political constraints and all available information must be brought to bear.

5. Set Ambient Environmental Quality Objectives and Standards

Some pollution control and environmental management strategies are based on water quality (or environmental quality) criteria. Such strategies relate directly to the quality of water, biota or sediments that must be maintained for a designated use. Biological assessments are conducted to determine the maximum allowable inputs that will ensure the desired levels of environmental quality are met.

Environmental quality criteria may be numerial or narrative. They may be set on a site-specific/case-specific basis, a multiple use or watershed basis, or on a national or generic basis. Due to the paucity of environmental quality 'standards' in Canada an increase of activity in this area is expected.

6. Develop and Evaluate Waste Treatment Technology and Disposal Methods

Problem correction (and prevention) requires the development or assessment of waste treatment and disposal methods. In this regard biological tests can be useful

- to identify in-plant sources/causes of toxicity
- to compare wastewater treatment alternatives at bench, pilot or full scale
- to evaluate wastewater treatment systems for toxicity reduction efficiency
- to identify and screen potential problems in solid or sludge residues or residue leachates
- to assess residue treatment and disposal alternatives.

Biological test use for this purpose could be split into three levels of intensity: 1) that required for problem identification/screening; 2) that required for problem assessment or evaluation; and 3) that required to pass regulatory standards.

7. Develop and Apply Site Specific Controls

Site specific control measures and remedial actions are developed, derived and applied with information gained during problem assessment activities (above). Site specific control measures are linked more closely to desirable levels of environmental quality. They allow a solution to be tailored to a problem. This may be advantageous when the problem is unusual or unique, or when it is desired to attain a greater level of protection than that specified in a 'blanket' standard, or where mitigative measures can be planned into new developments, or in situations where an application/permit type of control system allows a case-by-case assessment.

A Note on Decision-Making Hazard Assessment Control Schemes

Various hazard assessment schemes based on toxicity testing and chemical analysis are being developed and evaluated for use in situations where the general need for control measures is recognized and the authority for control exists but a case-by-case assessment is required. This mechanism also allows some degree of site-specific control with 'national' uniformity. Such schemes can expedite systematic decision-making and remove the mystique attached to biotest results interpretation. A decision tree type structure is often utilized. Level of protection criteria are pre-established.

For C&P, this application of biotesting extends to international and multiagency initiatives on common problems and to assisting OGDs in complying with their environmental protection obligations. Examples of current efforts include those for: dredging and disposal of sediments in the Great Lakes; transportation of hazardous wastes (TDGR); dredging and disposal of sediments in the St. Lawrence; dumping of substances at sea (ODCA); gas plant sludge pond decommissioning.

8. Develop and Apply a 'National' Level of Protection

It is necessary on a national basis

- to expedite and ensure equitable and consistent control measures for the long-term protection of the Canadian environment
- to provide industry, government and the judiciary system with a clear understanding of intent, both in the letter and spirit of the law.

Such guidance or control measures may allow site specific 'tailoring' or impose blanket standards across the country; they may be aimed at industrial sectors or types of contaminants. National protection 'standards' may be defined in regulations, guidelines, codes of good practice, etc., the development of which may involve many of the activities described in problem assessment and control and associated biological tests and procedures. Generally, however, the subsequent recommendations and controls would contain a much smaller number of biotests for use. In its most formal application, a biological test and result may be defined and applied in a legal sense. That is, it may serve as a regulatory standard, as a prescribed and controlled parameter, e.g. the rainbow trout acute lethality test.

9. Enforce Regulatory Standards

The results of biological tests are sometimes utilized (or intended for use) in enforcement activities. This practice presently appears to be restricted to Section 33 of the federal Fisheries Act, and can be summarized as

- Conduct legal tests on regulated effluents for the purpose of prosecuting violations of the Fisheries Act regulations.
- Provide evidence or specify a "deleterious" effect from unregulated discharges or spills according to the general provisions of the Fisheries Act.

There is often a requirement to establish liability and aid courts in sentencing violators. This can involve activities other than a demonstration of lethality, e.g.: using sublethal and chronic tests to show potential impact; establishing linkages with real resources, indigenous species, the receiving environment; documenting actual impact by field biomonitoring.

The use of biological tests in the enforcement of other environmental legislation appears minimal although there is the potential for greater use in future, such as under the Ocean Dumping Control Act and the Environmental Protection Act (in draft).

10. Monitor Compliance to Control Measures

Biological testing is performed for the purpose of determining compliance with pre-established legal conditions. It could also be considered in the less formal context of monitoring compliance with guidelines.

Current efforts in compliance monitoring are primarily related to the regulations and guidelines of the Fisheries Act (or surrogate OGD/provincial legislations), wherein toxicity is a specified and controlled parameter.

Future efforts in compliance monitoring may increase with the development of additional control measures with biological tests, such as those for offshore exploration and production activities, dredging activities in the Great Lakes/freshwater in general, ocean dumping, hazardous waste transport. Biological testing may thus increase, where necessary, to monitor/audit compliance to new, modified or consolidated C&P legislation and guidelines or by intergovernmental agreement.

11. Evaluate the Effectiveness of Control Measures and Remedial Action

Biological testing is conducted to address the question of adequacy - how well present controls and remedial actions are protecting the environment. This information is an essential feedback to assess earlier decisions and modify them accordingly, and has been identified as one of the key elements of the framework for cradle-to-grave management of toxic chemicals (DOE 1986b).

Current activities include:

- assessment of receiving environment quality in order to evaluate existing national or site-specific control measures in preventing environmental degradation;
- documentation of deficiencies in the toxicity data on existing regulated effluents, (particularly where environmental criteria did not play a strong role in the definition of control measures) for input to or to stimulate the re-evaluation process;

- monitoring and evaluation of on-going remedial action plans for _in situ_ pollutants, contaminated sites, accidental spills, etc.
- verification of pesticide registration data predictions on effects to non-target organisms or in Canadian conditions, and completion of deficiencies in registration packages for input to the re-evaluation process.

A related future need and use of biotests is foreseen in the assessment and evaluation of ambient environmental quality 'standards'.

12. Monitor Long-Term Environmental Trends

Since environmental effects may occur very slowly and subtly, it is important to monitor long-term changes or trends related to the fate of contaminants or the general health of the environment. This information may be used to evaluate the adequacy of environmental quality 'standards' or the effectiveness of control measures or to identify new problems which require new control measures.

A Note on Human Health-Related Effects

In these times of new chemical abundance, considerable concern exists about the effects and risk to humans from the use and disposal of toxics. The role of DOE in this regard is not always clear with respect to the role of Health and Welfare Canada and would appear to benefit by further definition. While Health and Welfare has an obvious primary mandate, DOE likewise has responsibilities to ensure the health of the environment, particularly insofar as it may affect humans. It is not unreasonable to assume that DOE should be employing tests which are indicative of environmental health and monitoring for potential human effects. This is particularly true on those environmental samples which DOE commonly handles and are more experienced in and for which Health and Welfare has less interest.

Historically, monitoring for human health concerns from toxic chemicals has been limited in EP. More recently, increased activity and interest has been shown by EP managers, e.g. genotoxic testing, tissue disorders in endemic species. Greater efforts by C&P are forecast in the future use of biological tests to identify potential risk to human health from toxic substances.

3.4 Interactions between the Draft Environmental Protection Act and Biological Testing.

On June 26, 1987 the Canadian Environmental Protection Act (CEPA) received first reading in the House of Commons. Both the spirit and letter of the Act demand the use of toxicity tests and biomonitoring procedures, although the extent of that use remains to be clarified. Some potentially relevant elements of the Act are identified below.

Part I: Environmental Quality Objectives, Guidelines and Codes

Section 7 provides the Minister with general authorities related to; monitoring of environmental quality; the conduct of research and studies related to aspects of environmental pollution, including control and abatement; and the reporting and publication of same. The provision is very broad, as is the scope for use of biological tests and procedures. The major applications, discussed in Chapter 3.3 of this report, would include problem identification and assessment, evaluation of treatment technology, determination of environmental trends and changes and the effectiveness of control measures.

Section 8 requires the Minister to formulate environmental quality objectives and guidelines, release guidelines and codes of practice with respect to all components of the environment, to the release of substances and to activities that may affect the environment. These national 'standards' (objectives, guidelines or codes) would be related to desirable levels of environmental quality and to environmentally sound operational practices.

There is a close dependency between the above activities and biological testing. The latter is a tool used in both the development, application and evaluation of environmental 'standards'. Likewise the selection of biological tests, and the design of biomonitoring programs is very dependent on the quality - the 'level of protection' selected for the environment, and on the manner in which those 'standards' are expressed.

'Standards' for environmental quality may be expressed in both narrative and numerical form and in such a way as to prevent or control problem substances. Numerical criteria can be chemical-specific (in which case biological toxicity and effects data and/or technological capabilities would be used in the derivation) or a toxicity value itself can be defined as a discrete parameter. Narrative standards generally describe more qualitative goals and uses which also incur the use of various biological procedures to assess, monitor and pass judgements, thereon.

Part II: Toxic Substances

The Act provides broader powers to gather information which may allow control of chemicals already in use in Canada.

For the purpose of assessing whether a substance is toxic or is capable of becoming toxic (Section 14), the Minister may a) collect data and investigate the nature, presence, environmental effects, persistence (degradability), bioavailability and genotoxicity of substances, methods for testing the effects and methods for controlling its presence (technology development assumed), b) evaluate and publish the above, and c) recommend control measures. All of the above implies a need and desire for scientific knowledge and testing capability within the Department.

Where the Minister has reason to suspect that a substance is toxic, then the Minister can require that the responsible parties conduct specified tests (Section 17(1)(c)). Details are not provided on the type of information that would be requested under the Act; however, it is assumed that it would be consistent with that required for new chemicals (see below) and would include data on ecotoxicity. It is also logical to assume that data would be consistent with the environmental quality 'standards' established in Section 8 and data needs outlined in Section 14.

Section 29 requires notification prior to importation or manufacture of substances new to Canada and compels the submission of prescibed information. The Minister is obligated to assess the data to determine if the substance is toxic and is empowered to permit or prohibit use of the substance or request additional information or testing in order to assess toxicity (Section 32). Provision is made in Section 35 for regulations to prescribe the type of information, conditions, test procedures, etc. required in compliance of the above.

While the data requirements and testing procedures are not specified in the Act, other supportive documents and explanatory notes make strong reference to the Final Report of the Environmental Contaminants Act Amendments Consultative Committee (ECAACC). Guidance contained therein recognizes the international efforts through OECD, the identification of a minimum premarketing set of data (MPD) for a first assessment of new chemicals and an agreement to flexible implementation of the MPD principle. The report suggests a modified MPD for Canada which includes requirement for ecotoxicological testing (see Appendix 4B). More recently, Sharp (1987) provides a draft of proposed regulations governing new chemicals notification The regulations, based upon recommendations of ECAACC, specify the provision of information including "mutagenicity test data; ecological data, including fish and daphnia LC_{50}, biodegradation and bioaccumulation". The ECAACC recommendations also recognize (as does the Act) that the federal government should have flexibility to require additional information. It is assumed that additional toxicological information would be consistent with the MPD recommended by OECD (see Appendix 4B), the OECD guidelines for testing of chemicals (Appendix 4C) and with the data needs outlined Section 8 of the Act.

Subsection 47 allows for regulations which would require persons, who produce or import fuel, to submit prescribed information on the adverse effects on the environment. Again, the exact nature of the information is unknown.

The request of toxicological data from industry, its assessment by the Minister, and subsequent decisions rendered have several important implications to the future of biological testing capabilities, use and expertise in C&P.

1) The Minister should have staff who are intimately familiar and experienced with various ecotoxicological tests so as to
   - ask for the right information
   - assess its quality
   - interpret the results
   - determine environmental hazard
   - recommend appropriate controls.

2) The Minister should establish the criteria used in defining hazard, in evaluation of the submitted data packages and in setting controls, i.e. the level of protection provided to the environment.

3) The Minister should implement a system of quality control and assurance over industry supplied data, e.g. lab certification programs, government audit, conditions of Good Lab Practice (GLP).

4) The Minister should develop and/or select specific biological testing protocols and guidelines.

It must also be recognized that industry, private and government facilities will require lead time to prepare for the provision of necessary services.

Part IV: Federal Departments....
Provision is made in Section 52 and 53 to establish environmental protection guidelines for federal departments, boards, agencies or crown

corporations and environmental regulations for federal lands, works and undertakings in the absence in other legislation.

As discussed earlier in this report, biological tests may be used in the development, definition and evaluation of in-house regulations and guidelines.

Part VI: Ocean Dumping

The Ocean Dumping Control Act has been consolidated into the CEPA, linking it with the life cycle control of toxic substances. Biological tests and data, which were always considered important before, should play a stronger role in implementation of the provisions. That role would focus on the determination of specific permit conditions, as well as screening and setting priorities, improving the assessment methodology and process and evaluating the effectiveness of previous control measures.

Under the Act, the applicant must provide information as prescribed by the Minister, who may also conduct investigations in support and pursuit of information required to determine whether to grant a permit. As to the type of information, it is specified that the Minister shall take into account the factors in Schedule III. Those factors include: toxicity, persistence, bioaccumulation, effects of other dumpings and possible effects on marine life and use of resources.

Part VII - General (Enforcement)

The use of biological tests and procedures will be necessary for enforcement. A variety of offences of Part II (Toxic Substances) and Part VI (Ocean Dumping) of the Act are directly related to ecotoxicological testing and data.

For example, it is an offence to fail to provide information, to make false or misleading tests or provide the Minister with false or misleading information in purported compliance. Since those information requirements do contain ecotoxicological elements, the Minister would be expected to conduct such testing in order to detect and demonstrate failure in compliance or fraudulence.

Ocean Dumping without a permit or in contravention of permit conditions is an offence. In such situations ecotoxicological data is important in establishing the seriousness of the crime and would directly influence sentencing.

Section 107 sets severe punishments for polluting actions that are a crime against the environment. The offences are described in narrative terms. It is anticipated that prosecution of such cases would draw extensively on all types of ecotoxicological and biomonitoring data.

3.5 Recommendations

1. It is recommended that the C&P senior management endorse a national policy on the use of biological tests and procedures in environmental protection and management activities and that such a statement provide clarification on the role of biotests in various use categories and with respect to the attainment of desirable environmental quality.

2. It is recommended that LMC seek clarification on issues related to the new Environmental Protection Act such as may reflect on EP laboratories or C&P integrity, e.g. biotest protocols, quality assurance, pass criteria, legal actions, etc.

4.0 AQUATIC BIOLOGICAL TESTS FOR EP AND C&P

To satisfy client needs for sound environmental protection and management, C&P must utilize the results of biological tests and procedures which are conducted both in the laboratory and in the field under controlled conditions of exposure and in the field under the natural in situ conditions of exposure (described herein as toxicity testing capacity and a field biomonitoring capacity, respectively).

4.1 Selection of National Core Aquatic Toxicity Tests

Aquatic toxicity tests are defined as those which utilize captive organisms in 'controlled conditions'. These tests may be made on single chemicals, effluents and receiving waters, or combinations thereof, in permanent or mobile labs. Most of the tests would be performed by experienced staff of a toxicology laboratory.

It is recommended that nationally, EP should establish, maintain and apply for environmental protection, a 'core' set of aquatic toxicity tests. The set of 'core' tests outlined in Table 1 were selected as a 'best fit' for the various criteria set forth, and from the many acceptable tests available. The selection was made at a workshop of the Intergovernmental Aquatic Toxicity Group in November 1986. Criteria used in the selection process are discussed in Chapter 4.2. Additional explanatory notes follow Table 1.

Definitions of tests were only made to a level consistent with existing knowledge and confidence in available methodology. In most cases further refinement is required in selecting the exact protocol (Chapter 7). The recommendations are not meant to stifle progress on the development of other procedures for assessing aquatic toxicity (see Chapter 9). It is not meant to inhibit the use of other tests by operational centres with additional or specialized needs (see Chapter 4.3) nor the use of field biomonitoring procedures (see Chapter 4.4).

Table 1. **National Core Aquatic Toxicity Tests for C&P Laboratories**

Test Type/Response	Test Species
Lethality[1]	
freshwater fish - mortality	Salmo gairdneri (rainbow trout, kamloops, steelhead)
estuarine/marine fish - mortality	Gasterosteus aculeatus (3-spine stickleback)
freshwater invertebrate - mortality	Daphnia magna (water flea)
marine invertebrate - mortality	Artemia sp. (brine shrimp)
marine invertebrate - mortality	[Rhepoxinius abronius (amphipod)]
Sublethality	
freshwater fish - ATP stress (physiological)	Salmo gairdneri
freshwater fish - larval growth inhibition	Pimephales promelas (fathead minnow)
freshwater invertebrate - reproduction	Daphnia magna/Ceriodaphnia reticulata[2]
freshwater algae - growth inhibition/stimulation	Selenastrum capricornutum or others
bacteria - luminescence inhibition (metabolic)	Photobacterium phosphoreum
Genotoxicity	
bacteria - Dark Mutant Test/SOS Chromotest/Ames Test[3]	Escherichia coli/E. coli/Salmonella typhimurium
Bioavailability	
freshwater and marine fish - bioaccumulation	S. gairdneri, G. aculeatus, P. promelas
marine mollusc - bioconcentration	[Macoma balthica (clam)]
Biodegradability	
mixed aerobic bacterial culture (OECD series)	

1. includes incapacitation/immobilization
2. species to be addressed in protocols
3. alternative tests for indicating genotoxicity

[ ] denotes tests and/or species being evaluated for potential use in Ocean Dumping Control

Explanatory Notes to Table 1

- the range/availability of the three spine stickleback was the determining factor in its selection as a marine fish. Salmon of Canadian coasts are of different species and of periodic availability;
- Artemia was selected as a salt water zooplankton over other marine larvae or copepods for practical reasons. It is recommended primarily as a problem indicator/screening test;
- the adequacy of Rhepoxinius abronius as a species most suitable for the amphipod sediment assay is being assessed for the Pacific coast (in 87/88) by the Ocean Dumping Control Program managers;
- the rainbow trout ATP energy stress test was chosen in part to expand the use of a primary test species;
- the fathead minnow larval growth test in Table 1 is the only test for which EP has no present capability. It is however a main chronic regulatory test of the U.S. EPA, and a protocol already exists, a sound data base is available and the use of other end points with this fish is being investigated. The fathead minnow is particularly suited to warm water and salinity-related problems and complements the rainbow trout which is deficient in these two aspects . In Canada, the fathead minnow occurs from the Saint John river (NB) drainage west through southern Quebec, all of Ontario and major portions of Manitoba, Saskatchewan and Alberta;
- both Daphnia and Ceriodaphnia are commonly used in the daphnid reproduction test. Assessment of species most suitable for Canada would be part of a protocol development exercise;
- the dark mutant test, SOS Chromotest and Ames test are all considered only as indicators/screens for genotoxicity. Both the dark mutant and the Ames test indicate that the substance in question had a mutagenic effect, i.e. the cells mutated. The Chromotest indicates that the substance triggered genetic repair regardless of whether successful cell repair, death or mutation occurred. It is anticipated that the dark mutant test will be commercially available in the fall of 1987. Before this time it is planned that EP Montreal will assess the

applicability of this test. The Chromotest has been on the market for a few years now. Both the dark mutant and Chromotest are more practical than the Ames test. They require no culture maintenance and can be done in about one-tenth the time. The Ames test is the oldest and most familiar of the three genotoxic screens. An Ames-type test was recommended for use in Canada as part of a minimum test requirement for chemicals at even the lowest level of concern (Advisory Committee on Mutagenesis 1986).

- *Macoma balthica* is an infaunal bivalve found on three of Canada's coasts and potentially suitable for sediment phase tests required for the ocean dumping control program.

4.2 Criteria Used in the Selection Process

The set of core aquatic toxicity tests contains useful and acceptable procedures and embodies sound principles of ecotoxicology. The criteria considered in the selection process are described below.

Applicability.

The set of tests

- is broadly applicable to a wide range of environmental protection activities
- satisfies national legislation and perceived national role/high priority applications.

Legislation given particular considerations was the federal Fisheries Act, the Ocean Dumping Control Act (see Appendix 4A) and the draft Environmental Protection Act (see Chapter 3.4 and Appendix 4B). Needs defined from past, present and future applications described in Chapter 3.0 were used in the selection processes. Emphasis was placed on those needs judged to be of higher priority to EP nationally, considering the traditional role of EP (end of pipe focus) and the cradle-to-grave philosophy for toxic chemicals. Greatest importance was attached to elements of the 'control' category, in particular enforcement, and the development and application of national standards and waste treatment/disposal technology.

Acceptability.

The tests are recognized and used by other agencies and international organizations. The types of tests selected are as consistent as possible with

- OECD guidelines
- EPA regulatory use
- MISA future use
- ASTM methods.

The set of tests includes those in the OECD minimum pre-marketing data set and guidelines (see Appendix 4B and 4C) on aquatic toxicity tests for chemicals (which may be utilized in the new Environmental Protection Act). The selection includes several key tests proven and recommended by the U.S. EPA for use in a regulatory context. They have recommended methods for measuring acute toxicity with 23 freshwater organisms and 27 marine organisms (EPA 1985b) and four short-term chronic toxicity tests (EPA 1985c). Those organisms are listed in Appendix 4D and 4E respectively. Chapter 1.2 of this report outlines the U.S. EPA approach on use of toxicity tests.
The selection includes some of those tests being evaluated in Ontario MOE's MISA program (see Appendix 4G and Chapter 1.2 of this report).

The majority of the 'core' tests are well established with one or several protocols and a considerable knowledge base available from prior use to aid in the choice of suitable methodology and interpretation of results.

Toxicity Type.

The selection was made to ensure that the collective set provided an ability to:

(a) measure both acute and chronic toxicity

- acute toxicity is induced over a short exposure period to the toxicant, usually 96 hours or less with invertebrates and fish
- chronic toxicity is elicited after a relatively prolonged exposure and therefore often by lower concentrations than those which cause acute effects. Chronic toxicity is often associated with substances which are released continuously; persistent; bioaccumulate; or are genotoxic.

(b) measure lethal and sublethal effects

- lethality is measured by death
- sublethal effects are changes in an organism's behaviour, physiology, biochemistry, metabolism, growth, reproduction, etc. Bioaccumulation is also a sublethal response.

Test Organism Type.

The selection was made to ensure that the collective set incorporates several species and biological responses that account for the varied sensitivities of different trophic levels and for both freshwater and marine environments.

Practicality.

Where possible, the selection considered tests which are practical in terms of low maintenance, cost and difficulty and which have a quick turn-around time. Emphasis is also placed on this criterion for future test development. The majority of tests are currently being employed in at least one of the EP toxicity labs.

Relevant End Points.

Insofar as possible, the selection considered test end points which are measurable, conclusive, readily interpretable and ecologically relevant, e.g. mortality, reproduction, growth, etc.

4.3 Aquatic Toxicity Tests for Specialized Regional or Program Needs

In addition to the core set of aquatic toxicity tests, provision was made to identify specialized regional or program needs. Such tests would be required and justified on a case-by-case basis to accommodate:

- the need for results from indigenous species
- special programs or requests
- the different strategies employed by the EP operational centers or the different toxicological expertise at hand.

A strong preference was shown by the Pacific region for lethality testing on native fish species (Appendix 4H). That tendency is consistent with their priorities on demonstrating effects to local resources, setting site-specific control measures, and an active Fisheries Act enforcement program.

The Atlantic region likewise identified the need for testing indigenous species of fish and invertebrates, particularly those useful for assessing sediment-related toxicity (Appendix 4I and 4J).

Quebec region labs, in line with their expertise, focused on micro-scale tests and on those which broaden the applicability of tests/species already in use (Appendix 4K).

Ontario region identified the need for additional biological tests to be used in characterization of Great Lakes sediments (see Appendix 4L).

4.4 Field Biomonitoring Procedures

Field biomonitoring procedures are defined as those which measure the actual distribution and effects of toxics in and on the receiving environment, where conditions of exposure are generally 'uncontrolled'.

Field biomonitoring procedures include the use of caged organisms in situ to measure either bioaccumulation, other sublethal or lethal responses, including some of those in Table 1. Likewise, individual animals of receiving environment populations may be sampled and examined for changes in functional processes (e.g. physiological, morphological) or analyzed to assess the bioaccumulation of individual substances. Included in field biomonitoring are classical studies of species diversity and abundance termed structural biomonitoring.

Field biomonitoring procedures for C&P activities are only described in general terms. Further specificity was considered to be beyond the scope of this study. It is noted that a NWRI/WQB National Aquatic Biomonitoring Committee was recently formed to define the role of biomonitoring in IWL water quality monitoring and to identify scientific research needs. Such an initiative could be expanded to address C&P programs and needs in freshwater field biomonitoring. In another exercise, attempts are being made to ensure that EP goals for protection of environmental quality are met in the marine environment. Some EP regional offices are now investigating approaches to marine environmental quality (MEQ) monitoring.

Some general applications of field biomonitoring results are discussed in Chapter 3 of this report and summarized in Appendix 4M. There is considerable agreement that EP needs such information, in order to interpret the significance of toxics in the environment and to set appropriate protection standards. However, a diverse opinion exists on the role of EP in the procurement of this information, particularly in light of the very limited resources EP has for such activities. An obvious solution to help alleviate the situation is to strengthen linkages with IWL and CWS and increase the level of program support. An alignment of biomonitoring issues, objectives and activities is considered both practical and necessary for C&P.

4.5 Recommendations

1. In support of a national policy, it is recommended that nationally EP should establish, maintain and apply a balanced set of well defined aquatic toxicity tests.

2. It is recommended that the core set of aquatic toxicity tests as defined in this report be adopted as a starting point for recommendation 1.

3. It is recommended that the LMC seek endorsement from senior management on a policy with regard to 1.

4. It is recommended that the product of IWL and MEQ initiatives to define field biomonitoring procedures and usage, should be a result of joint efforts (EP, IWL, CWS) and reflect overall needs of C&P.

5.0 AN AQUATIC TOXICITY TESTING CAPABILITY FOR EP AND C&P

5.1 General Perspective

A directional cue about in-house scientific capability and biological testing can be taken from a number of sources. For example, recent commissioned reviews (Macdonald, Nielson, Pearse) of DOE support the need for greater environmental invervention, for basic in-house research, for superior technical and scientific knowledge, and for improved internal capabilities in identifying, investigating and controlling problems. The Deputy Minister of DOE has stated that science and knowledge are the basis for our activities in the Department and central in a conceptual model of the Department's purpose of contributing to the management of resources held in common by all Canadians. In another study, ecotoxicological testing is considered an important component of the knowledge base in the long-term DOE environmental sensing strategy (DOE 1986c), particularly with respect to the intervention and early warning functions. Likewise, in a 'cradle-to-grave' approach to controlling toxic chemicals (DOE 1986b), the use of biological tests at certain stages throughout the life cycle of those chemicals is implied. Action elements of the draft Federal Environmental Quality Framework also indirectly identify the requirement for biological testing.

To implement the Environmental Protection Act DOE will require in-house capabilities and the use of biological tests with respect to providing nationally consistent standards of environmental quality and to investigating and controlling toxic substances (see Chapter 3.4 of this report). Emphasis is being placed on preparing a "compliance policy that will encourage industry to act in an environmentally responsible manner" and "enforcement against those who do not accept that responsibility".

The view of our Minister is that DOE will continue to play a very strong role in protecting the environment. The State of the Environment Report for Canada (1986) suggests some evidence of declining environmental quality which must be curbed with progress on the scientific front. Public opinion polls too show that Canadians are concerned, and they have been assured by the Minister that they will be kept informed of the country's environmental quality status.

The resolution of environmental issues is important to the government and people of Canada. DOE requires a stronger technical and scientific capability to conduct basic applied research, and to prevent, assess, control and report on environmental problems. Numerous national issues require immediate attention and the Department faces an ever-growing workload. But there is 'little financial flexibility'; new resources will be an exception. The decision of where to apply the limited resource base becomes critical and the question of in-house testing capabilities becomes controversial. Yet, an in-house aquatic toxicity testing capability is concluded to be both essential to DOE and consistent with public expectations and with Departmental responsibilities and initiatives. It is also recognized that a balance must be maintained between the federal, provincial and private sectors with regards to responsibilities, expertise and facilities for toxicological testing.

Some specific and very basic reasons for EP to maintain an aquatic toxicity testing capability are summarized below.

5.2 Some Reasons for In-house Aquatic Toxicity Testing Capability

Audit of Federal Standards

The 'polluter pays' principle has always been in effect and that philosophy continues with increasing emphasis being put on self-monitoring. However, it is necessary to audit those activities/data to ensure that what is required is completed correctly. This applies not only to industry but to other federal and provincial bodies. Although there is no definitive policy on this issue, it is the opinion of this author that the final responsibility to audit compliance with federal environmental legislation must be within the Department and that certain audit analyses be conducted in-house.

Enforcement

Testing for potential purposes of legal action is an in-house activity. Direct control can be exercised over the operations and personnel. The overall integrity of data is more defensible in court and government officers only are involved in enforcement, sampling, testing and testimony. Considerable controversy has existed about the lack of a clear enforcement policy and inconsistencies in enforcement. This issue is currently being reviewed by the Fisheries Act Compliance and Enforcement Policy task force.

Quality Control and Assurance

There is currently no mechanism to ensure quality of private sector generated aquatic toxicity test data. Due to the recent trend in Canada and worldwide toward self-monitoring, industry and their consultants will be generating an increasing amount of toxicity data in compliance with government requirements.

QA/QC of aquatic toxicity tests which C&P is promoting in regulatory programs and upon which environmental quality are based, is considered an essential duty requiring in-house facilities (see also Chapter 8).

Methods Development

While officially considered an inappropriate activity within EP, many managers agree that basic in-house capability is required to conduct practical and applied research in order to improve test methods for prediction, detection and assessment of toxic effects. Protocol development is required for the regulatory use of tests and quality control purposes. Sensitive work can be conducted in-house relatively unbiased of outcome and with EP motives in mind. It may be that DOE has special facilities or expertise not available elsewhere or is able to conduct work others are ill equipped to undertake. It should be noted that external methods development can be extremely expensive, unproductive and difficult to manage.

Credibility and Internal Expertise

An internal testing capability increases the credibility and perceived authority of C&P. There is little doubt that it contributes to the development and availability, training and retention of skilled staff and technical expertise, which the Department relies on in its programs.

Program Delivery

In-house facilities help to improve the quality of program delivery by exposing project and policy managers to the use of biological testing and by providing staff with a more holistic experience and perspective in problem solving. There are benefits in the ease of priority setting and in being able to key capabilities to program requirements. It would also be unacceptable for C&P not to have an operational capability for using environmental protection and management tools which are recognized worldwide.

As discussed in earlier sections, the need and potential applications of biological tests and procedures are many and obviously beyond resource allocation possibilities. It is therefore suggested that aquatic toxicity testing capabilities be applied to the current EP priority areas of:

- identification and investigation of toxic chemicals hazards
- development and evaluation of waste treatment technology and disposal methods
- development and evaluation of national 'standards' of environmental quality and protection
- audit and enforcement of regulatory standards.

5.3 Recommendations

General

1. It is recommended that EP should establish and maintain a national in-house capability to conduct the core aquatic tests outlined in Table 1 with appropriate protocols and a quality assurance program.

It must be recognized that any description of capability can not be rigid and must reflect advancements in toxicology techniques and the C&P role as it evolves, e.g. under new legislation, clarifying policy statements, definitions of environmental quality.

Capability is defined herein only in terms of types and quality of service (tests) and not in the level or quantity. The resources attached cannot be precisely defined because they are a function of the workload and priorities which are controlled and assigned on a fragmented basis. As mentioned earlier, the problem is not so much the lack of need for toxicity testing but rather being unable to quantitatively project that need. It is almost paradoxical that while the needs appear to grow, the level of support remains the same or shrinks. It can only be said that if C&P managers are serious about their programs and maintaining in-house aquatic toxicity testing facilities then they must provide resource support to these laboratories to ensure their needs and national requirements are met and a viable level of service is maintained.

2. It is recommended that C&P laboratory activities be integrated/-coordinated with respect to in-house aquatic toxicity testing services, research and methods development.

This would primarily be between EP and IWL programs, to facilitate the conduct of cooperative projects, the utilization of each other's facilities and expertise and effective research performance. Such activities are considered essential to the efficient use and survival of existing resources.

Distribution of Tests and Provision of Service

Present C&P in-house capabilities to conduct core toxicity tests are described in Table 2. A framework for the future distribution of core toxicity tests is suggested in Table 3. Guiding principles in that selection and the provision of service are:

- to have available nationally the core testing in-house capability so defined;
- to have a basic level of service in each primary lab;
- to have a practical geographic distribution of core tests;
- to have a reasonable balance of resource demands between labs;
- to reduce implementation costs by building on the existing facilities and expertise;
- to satisfy needs of operational centres without lab facilities and anticipated future use of toxicity tests in C&P and ultimately to satisfy the demands of a national policy.

With regard to the above, it is recommended that

3. Not all labs need have equal testing capability nor the complete set of core aquatic toxicity tests. Each lab should have a portion of the core set and might have additional toxicity tests for specialized needs. It should be noted that a testing capability for specialized regional needs can often be attained without substantial additional cost once core test facilities are in place;

4. All primary regional EP labs (Vancouver, Edmonton, Montreal, Dartmouth) should have or first develop the capability to conduct a rainbow trout acute lethality test, a daphnid acute lethality test, an algal growth inhibition test and a bacterial luminescence inhibition test. A minimal 3 PY allocation per lab was recommended by IGATG;

5. A similar core testing capability should be developed in both eastern and western Canada with each of the lab pairs, Vancouver/Edmonton and Montreal/Dartmouth, being able to complement each other in terms of freshwater and marine biases and core tests available;

6. All EP labs should provide service to all EP responsibility centres and cost recovery mechanisms established within a relatively uniform price structure.

7. A lead lab concept should be pursued to further provide service on more highly specialized tests or where the frequency of use does not justify

having those tests on-line. Additionally, a lead lab should be assigned responsibility for individual tests in terms of protocol and QA/QC functions for the core tests;

8. Suitable arrangements should be made for the Wastewater Technology Centre, Burlington, to obtain necessary in-house testing services in part or total from NWRI at Burlington. The test needs for WTC are currently defined as a genotoxicity test, daphnid lethality test, algal growth inhibition test and bacteria luminescence inhibition test, all of which are available at NWRI. Some of these tests are also offered by the private sector, which may be more suitable for routine large-batch analysis, provided a suitable QA program can be put in place;

9. The Ontario regional office should pursue the development of a suitable arrangement to obtain necessary in-house testing services from Ontario MOE (Rexdale) and NWRI (Burlington) and other existing EP laboratories. Additionally, it is anticipated that the private sector (with appropriate quality assurance) will respond to provide tests with Great Lakes indigenous species such as defined in Ontario E&P needs for characterization of Great Lakes sediments;

10. Industrial Programs Branch, Ocean Dumping Control Division, Environmental Emergencies Technology Division and Commercial Chemicals Branch should develop and/or obtain necessary in-house testing services from regional labs. If Ottawa area facilities are needed, the LMC and TDTSB should investigate the use of space at RRETC.

11. Secondary testing facilities should be supported where necessary for remote offices, e.g. St. John's, Whitehorse or for special situations or projects, where mobile lab, temporary lab or single test capability is required.

Note: At the time of this writing an assessment is in progress on the fate of the EP-Edmonton fish testing facility. The result of that decision may affect Tables 2 and 3, which assumes an existing and future capability in this regard. Should this change, then (1) the alternative facility shown for the fathead minnow test would be the only possible one, and (2) the EP-Vancouver lab would be the most logical source to service in-house fish toxicity testing needs of W&N region. The problem is, however, that the present facilities or the EP-Vancouver lab are regularly operated at full capacity and therefore are insufficient to handle a sustained external sample load.

Note: The ASTM protocol for marine and freshwater fish bioconcentration specifies the use of the rainbow trout and fathead minnow (freshwater) and the 3-spine stickleback (marine). Those species are all in the national core set recommended. For the purposes of Tables 2 and 3 it is assumed that the labs would adopt the protocol for those species which it maintains. The ASTM protocol also requires a flow-through system for exposure of the fish. All EP labs now have this system as a previous requirement for legal testing with rainbow trout. EP Dartmouth and EP Newfoundland also have flow-through marine system. EP Vancouver has running seawater in the facility used for holding marine organisms but not in the testing lab. Only static marine testing can be performed with trucked-in seawater.

Note: For the purpose of recommending additional capabilities and costing, a tentative selection of the dark mutant test was made from the three potential genotoxicity tests available. This assumes it receives a favourable technical evaluation. The present choice was made on the basis that training, maintenance, O&M, and capital are collectively lower than for the Chromotest or Ames test. The dark mutant test is run on a Microtox unit which should be in all four EP primary labs.

Table 2. Current C&P In-House Capability to Conduct National Core Aquatic Toxicity Tests WITHOUT Uniform Protocols or QA (as of January, 1987)

		EP - Vancouver	EP - Edmonton	EP - Montreal	EP - Dartmouth	EP - St. John's	EP - Burlington
Lethality							
trout - Salmo[1]	mortality	x	x	x	x	x	
stickleback - Gasterosteus	mortality	x			x	x	
waterflea - Daphnia	mortality	x	x		x		x
brine shrimp - Artemia	mortality			x	x	x	
amphipod - Rhepoxynius	mortality						
Sublethality							
trout - Salmo	ATP energy stress			x			
fathead minnow - Pimephales	larval growth						
waterflea - Daphnia/Ceriodaphnia	reproduction				x		x
algae - Selenastrum	growth inhibition		x	x			x
bacteria - Photobacterium	light inhibition	[x]	x	x			x
Genotoxicity							
dark mutant test	mutagenicity			(x)			
SOS Chromotest	genotoxic potential			x			x
AMES test	mutagenicity		x	x			
Bioavailability							
clam - Macoma	bioaccumulation				x	x	
freshwater and marine fish	bioconcentration	x	x	x	x	x	
Biodegradability							
mixed aerobic bacterial culture (OECD)				(x)			

[1] - includes initial QA/QC and standard protocol

( ) currently being developed at this facility in 87/88

[ ] part-time borrowed capability. Instrument owned by BC MOE

Table 3. Proposed Future Distribution of National Core Aquatic Toxicity Tests in Primary C&P Labs
WITH Appropriate Protocols and QA Programs.

		EP - Vancouver	EP - Edmonton	EP - Montreal	EP - Dartmouth*	IWL - Burlington
Lethality						
trout - Salmo[1]	mortality	x	x	x	x	-
stickleback - Gasterosteus	mortality	x	-	-	x	-
waterflea - Daphnia	mortality	x	x	++	x	x
brine shrimp - Artemia	mortality	++		x	x	-
amphipod - Rhepoxynius?	mortality	++	-	-	++	-
Sublethality						
trout - Salmo	ATP energy stress	++	(1)	x	(3)	-
fathead minnow - Pimephales	larval growth	-	++	(2)	-	-
waterflea - Daphnia/Ceriodaphnia	reproduction	(3)	++	(3)	x	x
algae - Selenastrum	growth inhibition	++	x	x	++	x
bacteria - Photobacterium	light inhibition	x	x	x	++	x
Genotoxicity						
dark mutant test	mutagenicity	++	++	X	++	
SOS Chromotest	genotoxic potential	(1)		x	(1)	x
AMES test	mutagenicity	(1)	x	x	(1)	
Bioavailability						
clam - Macoma	bioaccumulation	++	-	-	x	-
fish	bioconcentration	x	x	x	x	
Biodegradability						
mixed aerobic bacterial culture (OECD)				x		

KEY

x	existing test capability
++	suggested additional capability
(1)	alternative to chronic fish test or genotoxicity test shown
(2)	alternative facility for that test
(3)	future option to consider
-	not recommended
?	insufficient information for present assignment
*	potential division or duplication of tests between Dartmouth and St. John's was not addressed

Implementation

12. It is recommended that EP use a phased approach to move from the present to the proposed testing capability. 'Present to proposed' includes not only the addition of tests but bringing present capabilities in line with the protocols selected for Canada. It is recommended that systematic efforts on both protocol selection and implementation and QA/QC programs be supported and coordinated through the Lab Managers Committee.

An implementation ranking was assigned on a four level basis to provide some direction and priority in this matter. This option was developed by folding together all the factors and recommendations above (Distribution of Tests) and considering the status of protocols for the tests, i.e. the time and effort necessary to complete the selection thereof. Variations of what is suggested or other options are possible. That discussion would be part of the next step of developing a final plan and following feedback on this report. It should be noted that there is a potential for division or duplication of tests between EP Dartmouth and St. John's. This issue was not addressed herein.

13. It is recommended that the approach outlined below serve as a template to plan additional toxicity testing capability.

Level I:

- a Macoma bioaccumulation test for Vancouver
- a Daphnia lethality test for Montreal
- a Microtox for Dartmouth.

Level II:

- a Microtox for Vancouver
- an algae growth inhibition test test for Vancouver and Dartmouth (microplate technique currently assumed)
- a daphnid reproduction test for Edmonton
- a genotoxicity test for Dartmouth and Vancouver (dark mutant test currently assumed)
- an Artemia lethality test for Vancouver
- a dark mutant test (if evaluation proves favourable) for Edmonton.

Level III:

- an amphipod sediment assay for Vancouver and Dartmouth (in conjunction with Ocean Dumping Control Program managers evaluation)

- a rainbow trout ATP stress test for Vancouver.

Level IV:

- fathead minnow growth test for Edmonton
- optional ATP stress test for Edmonton and Dartmouth and daphnid reproduction test for Montreal and Vancouver.

14. Following general agreement on the above or modification to the satisfaction of the responsibility centres, it is recommended that a detailed schedule be developed for the individual labs to follow. It is recommended that the addition of new tests be carried out in conjunction with the implementation of selected protocols by the labs.

15. It is suggested as a target that Level I and Level II be scheduled for completion in or before fiscal year 88/89. Some of the associated activities can and should be conducted in 87/88. Level III should follow for completion in/or before 89/90. Level IV tests have as yet an unspecified time frame.

Alternatives

There are many possible variations to what has been recommended above although most would only have minor implications. The degree to which C&P laboratory services integrate could influence the distribution of testing services geographically but major changes to the types of tests are unlikely.

Total use of the private sector for toxicity testing is not considered a feasible option. It is contrary to the balanced position and the need for scientific capability advocated by many. It would be in opposition to basic reasons for in-house services already outlined. It would mean a damaging loss of technical expertise and scientific credibility and removal of EP from major existing roles and duties. Quality assurance on external contract laboratory data (which has problems now) would worsen (see Chapter 8.5).

Status quo also is not considered a wise choice. It would in effect ignore the very concerns which generated this report, i.e. the national inconsistency and lack of general acceptance in the use and quality of biological tests and the low level of resourcing. Responsible preparation for operational needs is required now in order to avoid greater problems and Departmental embarrassment in the future.

6.0 CORE TEST COST ESTIMATES

Suggested improvements to in-house toxicity testing services have included the addition of core tests (Chapter 5.3) and the selection and implementation of protocols (see Chapter 7.0). For many of the associated activities, the resource requirements are difficult to project because they are laboratory specific. It was considered most practical that a detailed schedule and resource implementation plan be developed by each lab following general agreement on the types and distribution of the various tests. Notwithstanding the above, some preliminary estimates and available cost data are provided.

6.1 Resources to Establish Additional Capability

The activities associated with protocol development, can proceed as a relatively independent component of the other activities required to establish additional testing capabilities. Protocol development costs are described in Chapter 7.4. The estimated level of resourcing for the strategy outlined therein is $50K O/M and 1 PY for a protocol development officer/coordinator. PY's as required for the protocol writing teams and in-house round robin testing are not specified.

The resource requirements to establish and offer the recommended additional toxicity tests can be considered in three activity stages:

1. Initial set up and staff training;
2. Initial QA performance checks;
3. Routine provision of service.

1. A preliminary estimate of resources for the initial equipment purchases and set-up, and staff training are presented in Table 4(a) and summarized in Tabel 4(b). It can be seen that those resource needs are surprisingly small since the basic lab infrastructure already exists. With regards to training, it is suggested that a sound practice to follow is to send staff for instruction to another

laboratory which already has experience in the test being put on-line.

2. No firm estimates are provided for the stage of initial QA and performance checks which must be conducted so that a lab can offer the test, to their clients, with confidence and understanding in the procedure and results. Such activities might include; testing with the various types of samples normally encountered and for which there is little existing information on use of that procedure; culture or stabilization of test organism stock; verification of inter and intra lab accuracy and precision. While the necessary resources are mainly PY related, the requirement is highly dependent on the test procedure, the lab and staff involved.

   Very crude estimates of the level of effort required for initial QA and performance checks are suggested in Table 4(a). The estimates assume no major start-up problems are encountered. They are based on the minimum number of samples/runs with each test procedure being: 10 each of Daphnia lethality, Microtox, algae microplate, brine shrimp lethality, dark mutant genotoxicity tests; 5 of the amphipod sediment assay; 3 of the trout ATP stress, Macoma bioaccumulation and Daphnia reproduction test.

3. Some information on the costs of maintaining and providing routine service for various biotests is described in Chapter 6.2. It also must be recognized to budget for the care and culture of additional tests species as required to establish additional capability. In addition, costs associated with quality assurance must be considered. It has been suggested that 10-15% of the operating costs in an established program go toward QA. Obviously the putting new tests or protocols in place would, incur a greater short term efffort than the situation of maintaining a routine ongoing QA program. The resources required in either case are predominantly salary and minor O&M supplies.

Table 4(a). Estimate of Resources for Initial Set Up and Staff Training Associated with Additional Recommended Toxicity Testing Capability

TEST	EP - VANCOUVER			EP - EDMONTON			EP - MONTREAL			EP - DARTMOUTH		
	CAP	O&M	PD	CAP	O&M	PD	CAP	O&M	PD	CAP	O&M	PD
Level I:												
Macoma bioaccumulation	-	1K	4									
Daphnia lethality							-	$2K^1$	7			
Microtox										25K	$2K^1$	7
Level II:												
Algae growth (microplate)	11K	$1K^2$	3							11K	$1K^2$	3
Brine shrimp lethality	2K	$3K^1$	5									
Daphnia reproduction				-	$3K^1$	20				-	$5K^1$	7
Genotoxicity (dk mutant)	-	$5K^1$	7	-	$5K^1$	7						
Microtox	25K	$2K^2$	7									
Level III:												
Amphipod sediment assay	1K	$3K^1$	8							1K	$3K^1$	8
Trout ATP stress	15K	$5K^1$	20									
Level IV:												
Fathead minnow growth				10K	$3K^1$	40						
Trout ATP stress (optl)				(15K)	$(5K^1)$	(20)				(15K)	$(5K^1)$	20
Daphnia reproduction ("")	-	$(3K^1)$	20				-	$(3K^1)$	20			

1: Includes travel O&M for one training trip
2: Travel O&M for training trip included with dark mutant test (Vancouver) or Microtox (Dartmouth)

Explanatory Notes to Table 4(a)

1. Capital

Microtox: refers to unit purchase price (analyzer, recorder, starter pack)

Chromotest: major item is multiscan colorimeter

Algae microplate technique: requires incubator and coulter counter.

Daphnia/Artemia: requires two incubators and stereoscope to run collectively lethability and/or life cycle tests. It is assumed that only Vancouver would require some additional equipment for these tests.

Trout ATP: requires ATP photometer

Fathead Minnow: requires 6 place balance

2. O&M

Includes O&M supplies for set up and practice plus travel for training purposes where indicated. Each trip assumed to be $1,500.00. Assumes 25 tests for practice of Microtox, Chromotest or dark mutant.

3. PD

Person days for set up and training time.

4. QA & PROTOCOLS

Assumes an existing complete protocol to follow but participation in quality assurance program is not included.

5. Alternate Genotoxicity Test

The SOS Chromotest as an alternate to the dark mutant, is estimated for use in Table 4 as 15K Capital, 4K' O&M, 7 PD.

Table 4(b). Preliminary Estimate of Resources for Regional EP Laboratories to Bring On-Line the Additonal Recommended Toxicity Testing Capability

LAB	LEVEL I & II TESTS			LEVEL III TESTS		
	Set-Up*	Check-Out**	Total	Set-Up*	Check-Out**	Total
Vancouver						
Cap	38K	-	38K	16K	-	16K
O&M	12K	0.6K	12.6K	8K	0.6	8.6K
PD	26	34	60 PD	28K	30	58 PD
Edmonton						
Cap	-	-	-			
O&M	8K	-	8K			
PD	27	29	46 PD			
Montreal						
Cap		-	-			
O&M	2K	-	2K			
PD	7	10	17 PD			
Dartmouth						
Cap	31K	-	31K	1K	-	1K
O&M	8K	0.2	8.2K	3K	0.2	3.2K
PD	17	6	23 PD	8	18	26 PD

* refers to initial set-up and staff training costed in Table 4(a)

** refers to initial QA and performance checks explained in Chapter 6.1

6.2 Estimates of Cost-per-Test

Qualification

The state-of-the-art on "costing" of biological tests is poorly developed. Determination of an accurate cost-per-test is complicated by many variables and the tendency is to revert to simple methods of deriving an associated dollar figure. Quality in general has a major influence; the nature of the QA, the protocol followed, the precision and reproduciblity, the statistical validity required, even the type of food the organisms are fed. A 96 hour LC_{50} can easily be obtained by methods which reflect an order of magnitude difference in effort and costs.

Caution should be exercised in the use of the cost data given here. They are estimates only and without complete qualification.

EP Toxicity Testing Services

Information on costs in three EP labs (Dartmouth, Vancouver and Montreal) is provided in Appendices 6A, 6B and 6C, respectively. It must be stressed that these were not derived in the same fashion and cannot be directly compared. The data could, however, be used to derive common billing rates for outside users (or within C&P). The material provided from EP Montreal provides a comprehensive breakdown of variables useful for planning and operational purposes. Estimates on tests not conducted in that lab, but which are part of the core set, were also derived for comparative use.

It is noted that overall the cost per test estimates between government and the private sector are comparable, although large differences do exist on the individual test basis.

Private Sector Costs

Cost to purchase toxicity tests in the Canadian private sector are presented in Appendix 6D. The data was obtained from several regions in the country, but only from labs with routine on-line capability. The results indicate that a large cost variance was related to location, most likely a function of market demand and competition. A major factor in cost variance between private sector labs (and between government and private labs) is quality. "You get what you pay for" is very applicable in this case. The exact methodology and QA attached to any analytical test has an overwhelming influence on effort and therefore dollars. Other items which influence prices are whether or not toxicity testing is a primary service or a subsidiary one, whether it is a 'loss leader', and the magnitude and stability of the workload. It was noted that the Canadian private sector had a limited capability to conduct the wide range of tests specified in the "core" set but was better equipped for rainbow trout acute lethality testing. Only three labs were found which offer wide spectrum service.

Appendix 6E presents cost data obtained from a survey of 26 labs in the U.S. EPA Region IV (Bill Peltier pers. com). Again, large variations were seen in the numbers for the reasons described above. Nevertheless, they do indicate relative costs for the various categories of tests which EPA puts into effluent permits and therefore requires industry to report on.

6.3 Recommendations

1. It is recommended that the LMC prepare a detailed cost implementation plan for the addition (over the next 3 years) and maintenance of testing capability outlined.
2. It is recommended that LMC establish standing cost-per-test charge out rates (either per each lab or per all labs) for external and internal (C&P) clients.

7.0 NATIONAL PROTOCOLS AND PROCEDURES FOR AQUATIC TOXICITY TESTS

(prepared by R. Scroggins, G. Sergy, R. Watts)

7.1 Introduction

At a one day workshop held in Moncton, November 10, 1986, federal members of the Intergovernmental Aquatic Toxicity Group (IGATG) selected a minimum national core of 14 aquatic toxicity tests for use in EP laboratories. At the annual IGATG meeting held the following day, the membership fully endorsed the national aquatic toxicity test core list (see Table 1: Chapter 4) and recommended that 'Canadian' protocols and procedures be developed for each test. Individual members of IGATG compiled a total of 70 existing Canadian and international protocols and procedures which related directly to the 14 national core tests.

In preparing for the writing of this chapter, all 70 existing protocols and procedures were reviewed. As part of this review, key methodology aspects of each test protocol and procedure were assembled (Appendix 7A) to provide a comparison of some of the similarities and differences. In Addendum 2 of this report, the protocols and procedures are bound in their entirety in 14 separate compendiums (ie: Addendum 2, Parts A to N). Copies of Addendum 2 have been sent to federal and provincial aquatic toxicity laboratories in Canada.

Presented in the remainder of this chapter is a strategy for the development of national core aquatic toxicity test protocols and procedures, along with a proposed schedule, associated costs and suggestions for the use of externally contracted firms for completion of the required tasks.

For the purposes of this chapter, the term 'protocol', used by itself, signifies 'protocols and/or procedures', which are two different levels in the hierarchy and validation of analytical methodology. Both levels may be required for the core tests, depending on their intended use. The term 'protocol' is the most specific name for a method. It correctly refers to a set of definitive directions that must be followed, without exception, if the analytical results are to be accepted for a given purpose. Typically, they are prescribed by an official body for use in regulatory processes. This type of use can likewise be anticipated in EP.

A 'procedure', consists of specific directions necessary to utilize a method. Many ASTM standard methods are in fact standard procedures, which describe a district adaption of a technique for a selected purpose. A procedure does not, however, describe every detail. Intentionally or unintentionally there is flexibility provided to the analyst which may account for inter laboratory variability. National procedures are required for all the core aquatic toxicity tests.

7.2 Protocol Development Strategy for Core Tests

The overall strategy for developing protocols and procedures for the 14 core tests is:

1) Upon completion of the review of Canadian and international protocols and procedures available for each of the core tests, a selection will be made of one of two protocol development approaches; fast-track or traditional track.

2) The first draft of a national testing protocol will be prepared by a designated protocol development officer with selective EP toxicity lab support, and then subsequently reviewed by IGATG and LMC members.

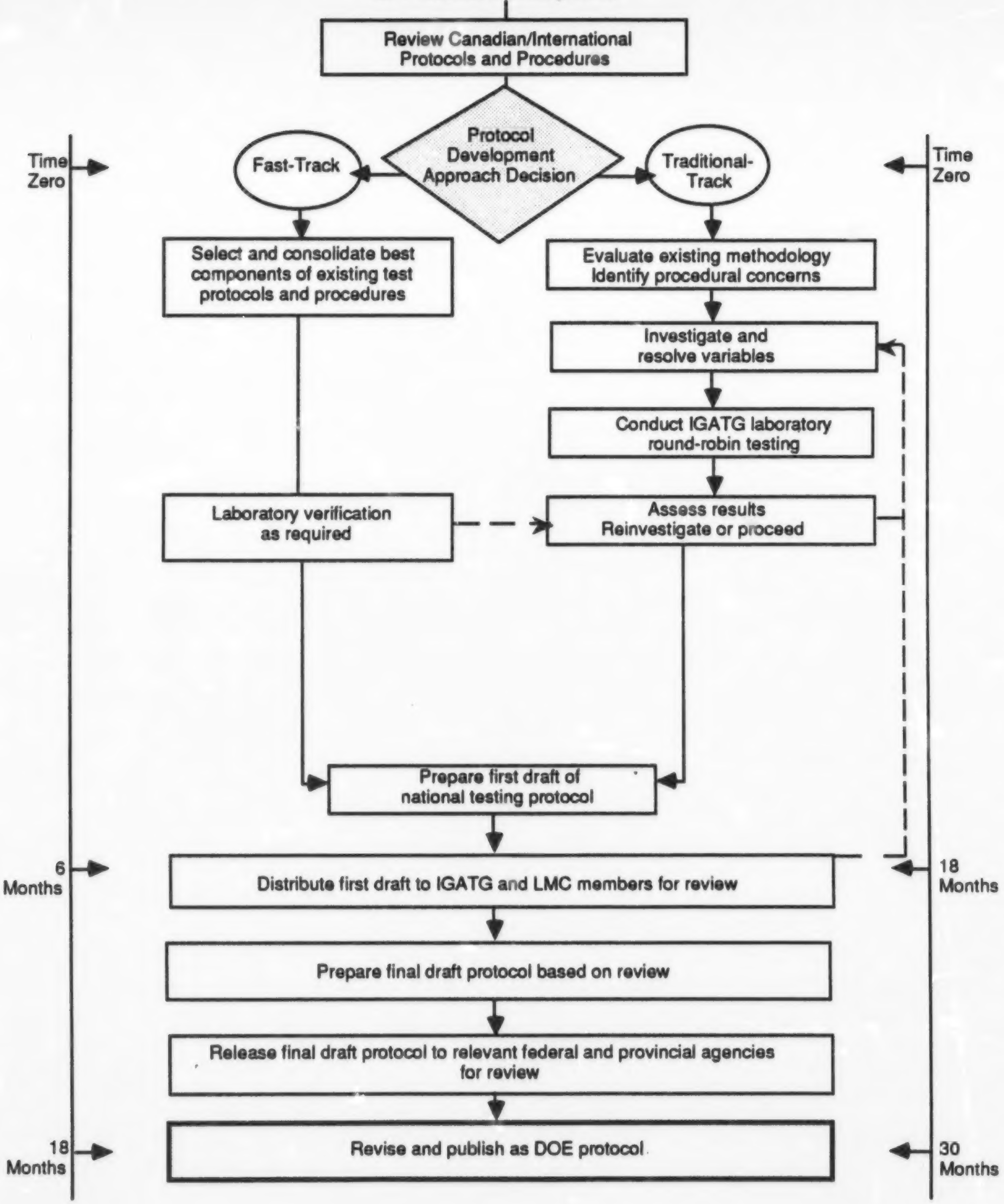
3) As the final draft national protocols are completed by either approach, copies will be circulated to relevant federal and provincial laboratories and departments for review and technical assessment.

4) After considering all review comments, the protocols will be completed and distributed as Environment Canada aquatic toxicity testing methodology.

The steps involved in developing a final draft national protocol by either the fast-track or traditional-track approaches are outlined in Figure 1.

An important component of the above strategy is the designation and resourcing for a protocol development project coordinator/officer (which

Figure 1: Steps in a Fast-Track and Traditional-Track Approach for Protocol Development


Review Canadian/International Protocols and Procedures
Protocol Development Approach Decision
Fast-Track
Traditional-Track
Time Zero
Time Zero
Select and consolidate best components of existing test protocols and procedures
Evaluate existing methodology Identify procedural concerns
Investigate and resolve variables
Conduct IGATG laboratory round-robin testing
Laboratory verification as required
Assess results Reinvestigate or proceed
Prepare first draft of national testing protocol
6 Months
18 Months
Distribute first draft to IGATG and LMC members for review
Prepare final draft protocol based on review
Release final draft protocol to relevant federal and provincial agencies for review
18 Months
30 Months
Revise and publish as DOE protocol

may be the same or two different persons). The protocol development officer would be primarily responsible for the technical writing of the draft protocols while the coordinator would have overall responsibility for managing and completing the various steps in the development process.

Based on the protocol development strategy schedule outlined in Table 6, the services of the project coordinator/officer will be required for a four year period.

A key component of the protocol development strategy are the approach and priority setting decision criteria. Five criteria have been established to support the decision as to which protocol development track should be followed for each of the core tests and the order in which protocols should be developed under each track. Those criteria are:

1) number of Canadian and international aquatic toxicity testing protocols and procedures available.

2) whether or not any Canadian or international inter-laboratory round-robin/calibration exercises have been conducted for the core toxicity tests.

3) current EP in-house capability to conduct national core aquatic toxicity tests (without national protocols or a quality assurance program in-place)

4) proposed future EP in-house capability to conduct national core aquatic toxicity tests (with national protocols and a quality assurance program in-place)

5) estimation of EP program/project requirements for individual core tests (based on the 1986 survey of EP aquatic toxicity testing needs which identified programs/projects which currently or potentially could use biological testing as a component of their work).

In order to be eligible for the fast-track approach, the core test being considered must meet minimum requirements established for the first two criteria listed above. These requirements are that:

- more than one test protocol or procedure is available for the core test (either Canadian or international) and
- at least one inter-laboratory round-robin/calibration exercise has been completed for the core test (either Canadian or international).

The last three criteria have been developed to assist in establishing the order in which national protocols should be developed under each track.

Based on how well the individual core tests fit with the five decision criteria (see Table 5), nine core tests were selected for protocol development using the fast-track approach. The five remaining core test protocols were selected for development using the traditional-track approach. The proposed order of core test protocol development for both tracks is outlined in Table 6.

Further support and explanation for the Table 5 decisions on a protocol development approach/priority are presented for each of the 14 core tests in the discussion which follows.

TABLE 5: **Selection of Core Test Protocol Development Approach and Priority Using Evaluation Criteria**

Core Test Aquatic Toxicity Testing Protocol	APPROACH AND PRIORITY SELECTION CRITERIA							PROTOCOL DEVELOPMENT APPROACH DECISION		ORDER OF PROTOCOL DEVELOPMENT DECISIONS
	Number of Protocol/ Procedures		Round Robin/ Calibration Exercises		CURRENT EP LAB CORE TEST CAPABILITY	PROPOSED EP LAB CORE TEST CAPABILITY	INDIVIDUAL* CORE TEST NEEDS OF EP PROGRAM/ PROJECTS (86/87)	Fast-Track (FT)	Traditional Track (TT)	
	Can.	Int'l	Can.	Int'l						
Acute Lethality Testing Procedures for Salmo gairdneri	10	7	Yes	Yes	4	4	D	Yes	-	FT-1
Acute Lethality Testing Procedures for Gasterosteus aculeatus	2	0	Yes	No	2	2	C	Yes	-	FT-3
Acute Lethality/Immobility Testing Procedures for Daphnia magna	0	9	Yes	Yes	3	4	D	Yes	-	FT-2
Acute Lethality Testing Procedure for Artemia sp.	0	1	Yes	Yes	2	3	C	Yes	-	FT-6
Acute Lethality Testing Procedures for Marine Amphipods	1	0	No	No	0	2	B	-	Yes	TT-3
Sublethal ATP Energy Stress Testing Procedure for Salmo gairdneri	1	0	No	No	1	2	D	-	Yes	TT-2
Sublethal Testing Procedures for Pimephales promelas	0	3	No	Yes	0	1	A	Yes	-	FT-9
Sublethal Testing Procedures for Daphnia sp./Ceriodaphnia sp.	0	6	No	Yes	1	2	D	Yes	-	FT-7
Sublethal Growth Inhibition/Stimulation Testing Procedures for Unicellular Green Alga	2	5	P	Yes	2	4	D	Yes	-	FT-5
Sublethal Testing Procedures for Bacterial Luminescence	2	3	P	Yes	3	4	D	Yes	-	FT-4
Genotoxicity Testing Procedures for Bacteria	1	4	No	Yes	2	4	D	-	Yes	TT-4
Bioconcentration Testing Procedures for Fish	0	6	No	Yes	4	4	D	Yes	-	FT-8
Bioavailability Test Procedures for Marine Molluscs	1	0	No	Yes	1	2	B	-	Yes	TT-1
Biodegradation Testing Procedures	0	8	No	No	0	1	B	-	Yes	TT-5

* due to the subjective nature of this criteria, the estimate of the number of EP programs/projects which currently or potentially require the use of individual core tests have been grouped into four categories:

A = 1 - 10 programs/project; B = 10 - 25; C = 25 - 50; and D = >50; P= round robin planned during 1987/88 fiscal year.

Table 6. **Order and Proposed Schedule for Development of National Core Test Protocols.**

PROTOCOL DEVELOPMENT ORDER	Year One	Year Two	Year Three	Year Four	Year Five	Year Six
Fast-Track Approach Protocol Development						
1. Salmo gairdneri acute lethality protocol	++----	---				
2. Daphnia magna acute lethality/immobility protocol	++---	-----				
3. Gasterosteus aculeatus acute lethality protocol	++---	-----				
4. Bacterial luminescence inhibition sublethality protocol	++	------	-			
5. Unicellular green alga sublethality protocol	++	------	-			
6. Artemia sp. acute lethality protocol		++-----	---			
7. Daphnia sp./Ceriodaphnia sp. sublethality protocol		++-----	---			
8. Fish bioconcentration protocol		++---	-----			
9. Pimephales promelas sublethality protocol		++	------	--		
Traditional-Track Approach Protocol Development						
1. Marine mollusc bioaccumulation protocol	+++++	++-----	--			
2. Salmo gairdneri ATP energy stress sublethality protocol	+++	++++-	-----	-		
3. Marine amphipod acute lethality protocol		+++	+++++-	------		
4. Bacterial genotoxicity protocol			++++++	++---	---	
5. Biodegradation protocol			++	+++++	------	-

KEY: ++++++++++++++++++++--------------------

Protocol Development Period	Review Period
++++++++++++++++++++	--------------------

FAST-TRACK

1. Salmo gairdneri Acute Lethality Protocol

During 1970's, the rainbow trout acute lethality toxicity test was incorporated into two federal regulations and five federal guidelines (under the Fisheries Act) (listed in Appendix 7A). However, differences exist between these documents, in the actual test specifications. This situation was recognized by the IGATG committee in late 1970's. To help alleviate the problem, IGATG members prepared and published, in 1980, a standard procedure for testing the acute lethality of liquid effluent using rainbow trout. The 1980 standard procedure attempts to provide a common methodology alternative to the five different pass/fail type acute lethality procedures outlined in the Fisheries Act guidelines. However, the exercise was somewhat limited in that the 1980 procedure did not deal with a number of important concepts and issues (ie: use of LC50, LT50 or toxic unit endpoints, the question of pH adjustment, pre-test filtering of effluent solids, continuous-flow testing requirements, etc.) Some of these were considered in the preparation of the 1984 "Guidelines on the Use and Acceptability of Oil Spill Dispersants". Presently, the 1980 standard procedure for rainbow trout acute lethality testing still requires revision to bring it up to the level of detail of international acute lethality protocols available for this species.

The strategy for developing a national acute lethality protocol for _Salmo gairdneri_ (rainbow trout, kamloops trout and steelhead) is to build upon the oil spill dispersant guideline testing procedure based on a review of Canadian provincial and international protocols and procedures available. Development of this protocol has been given first priority under the fast-track approach for two main reasons:

- the _Salmo gairdneri_ acute lethality test is the most widely used aquatic toxicity test in Canada
- a formal protocol would have to be available to properly address revisions to the toxicity test sections of our national Fisheries Act regulations and guidelines and in order to satisfy testing requirements of the draft Environmental Protection Act.

2. Daphnia magna Acute Lethality/Immobility Protocol

EP has been on the verge of producing a *Daphnia magna* acute lethality protocol for several years. Two inter-laboratory round-robin exercises were successfully completed by IGATG members in 1982 and 1984. These round-robins addressed the issues of loading density, use of standard dilution water, test duration and feeding of the test organisms during the bioassay. The round-robin exercises also confirmed the value of measuring two endpoints: mortality and immobility. Three of the international protocols reviewed (APHA, ASTM and The Netherlands) require acute lethality (LC_{50}) and immobility (EC_{50}) to be reported.

Although a couple of issues remain, the development of a national protocol for *Daphnia magna* acute lethality testing using the fast-track approach should be relatively straight forward. The best aspects of the nine international testing protocols and procedures available will be considered in the preparation of the final draft protocol.

It is anticipated that the *Daphnia magna* acute lethality test will be specified in regulations and guidelines of the Environmental Protection Act.

3. Gasterosteus aculeatus Acute Lethality Protocol

EP - Atlantic region and Fisheries and Oceans Canada have developed acute lethality testing procedures for the three-spined stickleback, *Gasterosteus aculeatus*. Both procedures were developed to help quantify the toxicity of waste drilling fluids discharged from offshore oil and gas platforms to the marine environment. A limited inter-laboratory round-robin exercise was undertaken during the development of the DFO procedure. The two toxicity testing procedures are quite similar as well.

Several additional facts favour the development of a national acute lethality protocol for the three-spined stickleback by the fast-track approach:

- Gasterosteus aculeatus is a common species found in Canadian water off the Atlantic, Arctic and Pacific coasts
- Vancouver, Dartmouth and St. John's EP aquatic toxicity laboratory staff are familar with the acute lethality procedure
- there is an urgent need for a recognized marine fish acute lethality test and the three-spined stickleback is the most obvious choice
- the procedure is very similar that for Salmo gairdneri
- it satisfies regulatory requirements

4. Bacterial Luminescence Inhibition Sublethality Protocol

The protocols and procedures collected and reviewed are all based on the Beckman Microtox™, which measures the reduction of light emission from the photo-luminescent bacterium, Photobacterium phosphoreum, when exposed to toxic substances. There are few differences between the various available testing procedures.

The Microtox™ procedure is a routine toxicity test used by the EP Vancouver, Edmonton and Montreal aquatic toxicity laboratories for screening liquid effluent and ambient samples. A four-trial inter-laboratory round-robin exercise (Microtox Interlaboratory Comparison Study) coordinated by Dr. Ansar Qureshi of the Alberta Environmental Centre was initiated in 1986. Three EP laboratories are participating in the round-robin which should be completed during the 1987/88 fiscal year. Anticipating the availability of resources in the near future for the Dartmouth laboratory's purchase of a Microtox™ unit and the wide use of the aquatic toxicity screening procedure in Canada, development of a national protocol based on the Microtox™ procedure using the fast-track approach is recommended.

5. Unicellular Green Alga Sublethality Protocol

Seven Canadian and international growth inhibition/stimulation toxicity testing protocols and procedures were reviewed. Although the recommended test species, test vessel, test duration and test endpoints vary (see Appendix 7A), there are a number of similarities. For example, Selenastrum capricornutum is a recommended test species in five of the seven protocols/procedures.

One of the main procedural differences apparent from the review was the use of a microplate test vessel rather than the more traditional Erlenmeyer flask test vessel. The microplate technique developed by EP Montreal aquatic toxicity laboratory has been demonstrated to be considerably more cost-effective than the standard flask technique. EP Montreal is organizing an inter-laboratory round-robin exercise using the algal microplate technique for the summer of 1987.

Although there are some decisions to be made on test procedures, development of a national protocol for unicellular green alga growth inhibition/stimulation using the fast-track approach is recommended.

6. Artemia sp. Acute Lethality Procedure

There is basically only one international protocol available for Artemia sp. nauplii acute lethality testing. However, this protocol is based on the consolidation of numerous previous procedures and was in fact developed in an international exercise much like the traditional track process suggested in Figure 1. The test is widely used in Europe and has shown excellent reliability and reproductivity in a couple of major inter-laboratory calibration exercises. The fast-track approach is recommended on the strength of the present Artemia Reference Centre protocol.

7. Daphnia sp./Ceriodaphnia sp. Sublethality Protocol

Six international chronic toxicity test protocols and procedures were reviewed; four recommended Daphnia magna as the test species and two recommended Ceriodaphnia dubia. Survival and reproduction endpoint measures were common in all six while growth effects was considered in four of the protocols/procedures. Both Ceriodaphnia test procedures had durations in the 7 to 8 day range while the test duration of the Daphnia procedures were 14 days or greater.

Five of the six test protocols/procedures have been finalized only recently as they have been prepared through a lengthy development process. The recommendation that a national protocol be developed following the fast-track approach is made based on the rigorous development process and technical detail provided in the available international protocols. The possibility should be evaluated of developing two national protocols, one for Ceriodaphnia and one for Daphnia, once a detailed assessment of the six protocols/procedures has been completed.

8. **Fish Bioconcentration Protocol**

Six international bioconcentration protocols were reviewed; one was developed by ASTM while the other five were recommended by OECD. Four of the OECD protocols specified the use of test species not found in Canadian waters (ie: southern catfish, zebrafish, killifish and Japanese carp). The remaining two protocols (one OECD and one ASTM) are quite similar and recommend the same three test species, all native to Canadian waters (ie: two fresh water species, Salmo gairdneri and Pimephales promelas and one marine species, Gasterosteus aculeatus). All three species fit well into the national core test strategy in that they are also the recommended species of four other proposed national protocols.

As the four main EP aquatic toxicity laboratories have diluter systems for running legal flow-through trout bioassays, they are equipped to run either the ASTM or OECD bioconcentration test procedure (both require a continuous-flow delivery system). On the strength of these two international protocols, it is recommended that the national fish bioconcentration protocol be developed using the fast-track approach.

9. **Pimaphales promelas Sublethality Protocol**

Only two U.S. EPA short-term chronic toxicity protocols were available for review. One protocol measures fathead minnow larval survival and growth over a 7 day test period while the other protocol measures embryo-larval survival and teratogenicity over an 8 day test period. Although relatively new, both protocols are widely used in the U.S. NPDES wastewater discharge permitting program. ASTM is developing an early life-stage *Pimephales promelas* testing protocol but it is still in a draft stage of development.

Development of this national protocol is recommended using the fast-track approach as the core test met the minimum criteria. However, it has been picked as the last fast-track developed protocol because none of the EP aquatic toxicity laboratories have any experience with fathead minnow chronic toxicity testing.

TRADITIONAL-TRACK

1. Marine Mollusc Bioaccumulation Protocol

Two bioavailability testing procedures were reviewed. However, only the procedure developed by the EP Dartmouth aquatic toxicity laboratory measures biaccumulation potential. The EP Dartmouth procedure is more of a sediment bioassay since burrowing clams (*Macoma balthica* or *Mya arenaria*) are exposed to contaminated sediment as part of the test. The ASTM test procedure measures potential bioconcentration of contaminants from the aqueous phase. No sediment is added to the test vessel in this procedure.

As this national protocol is being developed primarily for use by the federal Ocean Dumping Control Program, the EP Dartmouth bioaccumulation test will be the procedure developed via the traditional-track approach.

2. Salmo gairdneri Sublethality Protocol

Only one procedure was available for review due to the original nature of the toxicity test. The procedure was developed by Dr. Christian Blaise of the EP Montreal aquatic toxicity laboratory in order to measure sublethal effects in trout which have survived a 96 hour acute lethality bioassay. Sublethal effects are quantified by comparing the ATP energy level in muscle of exposed and unexposed trout.

This procedure provides for an additional endpoint measurement which is more sensitive than acute lethality. The traditional-track approach for national protocol development has been suggested as currently only the EP

Montreal laboratory has the expertise and equipment required to conduct the test procedure.

3. Marine Amphipod Acute Lethality Protocol

Only one testing procedure was available for review. Federal Ocean Dumping Control program managers are seriously considering this sediment assay as one of two or three biological test procedures for use in assessing ODCA dumping applications.

During the 1987/88 fiscal year, the ODCA program is evaluating the test procedure using the amphipod Rhepoxinius abronius, however, the testing is limited to the evaluation of specific Pacific coast industry-contaminated sediments. Future research is planned to investigate the adequacy of the Rhepoxinius abronius test procedure for the Arctic and Atlantic coasts.

National protocol development should proceed via the traditional-track approach in conjunction with ODCA program initiatives.

4. Bacterial Genotoxicity Protocol

The Ames Test is the traditional mutagenicity procedure and three different procedures were reviewed for this test. However, the traditional-track approach was chosen for development of this national protocol. Two relatively new genotoxicity procedures, the SOS Chromotest and Dark Mutant assays, offer significantly greater cost-effectiveness and sensitivity. The SOS Chromotest is conducted routinely by the EP Montreal laboratory. Both the EP Montreal and IWL Burlington aquatic toxicity laboratories will be evaluating the Dark Mutant procedure during the summer of 1987.

A round-robin/calibration exercise involving the Ames, SOS Chromotest and Dark Mutant assays will be required before one of the procedures can be picked for development to national protocol status.

5. Biodegradation Protocol

Eight OECD biodegradation procedures were reviewed. Currently, the Commercial Chemicals Branch of EP has selected the modified MITI test for further investigation and use in screening new chemicals. The necessary equipment has been purchased and will be located in the EP Montreal aquatic toxicity laboratory. An evaluation of the procedure will begin during the summer of 1987. If the results are promising, the traditional-track approach will be followed to develop a national biodegradation test protocol. NOTE: Development of a biodegradation protocol may have to be accelerated, in order to meet the needs of the Environmental Protection Act.

7.3 Role of Externally Contracted Firms

The level of involvement of externally contracted firms such as environmental consultants and university groups in the protocol development project is directly related to the annual level of O&M resources available to the project coordinator. Likewise, the level of consultant participation will affect the schedule of protocol development and/or work load demanded of lab staff. External firms could be involved in a number of activities. These include (in order of decreasing priority):

- coordination of inter-laboratory round-robin exercises and preparation of a report on the findings
- search of literature for additional test protocols and procedures (ie: contract would cover all 14 core tests)
- peer review: to coordinate independant non-government expert(s) reviews of final draft protocols and to address procedural problems identified
- at protocol implementation phase, to provide training seminars at federal and provincial laboratories for interested parties.

Detailed review of available core test protocols/procedures and preparation of interim test procedures/draft testing protocols are, however, the responsibility of the protocol development officer. The cost of contractual support/advice in these tasks has not been included.

7.4 Schedule and Resource Requirements for Protocol Development Strategy

A schedule for the proposed strategy is directly affected by the annual level of resources available to the protocol development project. A possible schedule has been outlined in Table 6 based on the assumed availability of resources suggested and support from C&P toxicity laboratories.

In the first four months of the project under the fast-track approach, only one protocol writing team will be active. The draft protocol produced will act as a blueprint to be followed for the other 13 draft protocols. Over the next year and a half, writing teams will be active developing the remaining eight draft protocols to be developed by the fast-track approach. All protocols produced by either the fast-track or traditional-track approach must go through a 14 month review period following submission of the draft protocol. A period, a final national protocol will be prepared based on the comments received and widely distributed.

Protocol development activities under the traditional-track will begin at the same time as fast-track activities. However, due to the inter-laboratory round robin/calibration testing component, the time frame for developing draft protocols under the traditional-track is one year longer than the fast-track. If the need is identified for a technical assessment/round robin exercise of draft protocols developed by fast-track, then this activity will be accommodated.

The proposed annual budget for the national protocol development project is $50K. Approximately $30K will be required for external contracting activities (ie: 3 contractor-run round robins per year which includes report preparation) while the other $20K will be used to cover the travel costs of the project coordinator and protocol team writers. A number of miscellaneous costs (ie: courier charges, printing costs, etc.) are covered in the $20K as well. The resources will be managed by the overall protocol development project coordinator.

7.5 Recommendations

1. It is recommended that Environment Canada protocols and procedures be developed and published for the national core aquatic toxicity tests defined.
2. It is recommended that a protocol development project coordinator/-officer be assigned. The estimated total time commitment of this person(s) is 12 man-months per year (ie: 1.0 PY).
3. It is recommended that an annual budget of $50K per year be allocated to develop the 14 national protocols (as per the proposed schedule, outlined in Table 6. Sources for these funds should include all responsibility centres who will benefit from the exercise including TDTSB, CCB, IPB, Ocean Dumping Control Program and C&P laboratories.

8.0 BASIC ELEMENTS OF QUALITY ASSURANCE IN ECOTOXICOLOGICAL TESTING

(Prepared by D. MacGregor and K. Doe)

8.1 Introduction

This chapter has been prepared to provide an overview of the basic elements of quality assurance (QA) in ecotoxicological testing. It should be considered as a companion to the document by Lawrence *et al*.(1983) on QA in chemical measurements, which was endorsed by the EPS Laboratory Managers Committee (LMC) at its meeting of 14 March, 1984.

The intended scope of this overview is all toxicity tests, bioaccumulation tests and biodegradation tests currently performed in or for Environmental Protection laboratories. In general terms, it should also be applicable to all such tests which will be developed for use within the next few years in or for these laboratories.

This overview on QA is comprised of: a background commentary on QA, with emphasis on recent developments in Environment Canada and in other agencies; an outline of what are considered to be the major elements of a QA program, together with brief comments on the reasons for the importance of these elements; a short subsection on the costs of QA; a separate discussion of QA as it applies to work which is contracted-out; and concluding recommendations.

8.2 Background

Although QA has been of concern to specialists since the beginnings of quantitative science, within recent times it has come into prominence largely because of the Industrial Biotest fiasco in the United States (Tokay, 1984). Prior to the discovery that a vast amount of data which had been employed for regulatory purposes was highly suspect, it had apparently been widely assumed that QA was a matter which was best left to the specialists. It is only gradually that awareness has grown that QA is part of the overall management process. Unfortunately, the growth of such awareness has not yet been highly visible within Environment Canada, since no department-wide policy on QA appears to exist. It is predictable that, as we enter a new era with the introduction of the Canadian Environmental Protection Act and its attendant regulations, the department's inattention to QA may lead to lost prosecutions because of possible legal shortcomings of our test data.

Before further discussion of QA, it is appropriate to provide a definition. Although such definitions are numerous, one which is highly appropriate has been provided by McDonnell and Balsillie (1982): "... an overall system of management and operational activities designed to ensure adequate performance of quality control. The objective of quality assurance is to provide data which are complete, precise, accurate, representative and comparable. The quality assurance program must be directed to all aspects of monitoring: network design, station siting, sampling, analysis, data storage and validation, and interaction with management".

It is equally appropriate to define quality control (QC). Also in the words of McDonnell and Balsillie (1982), this is: "a system of routine checks and procedures included in normal operations to ensure a quality product (i.e. data)".

The reader should note that a QA program, including its associated QC activities, assures that steps to maintain and improve data quality are in place and that the limits of uncertainty associated with the data are known. Absolute validation of data can be achieved, but this lies beyond the normal limits of a QA program and is more akin to the programs undertaken in national standards agencies.

At present there is no Department wide or C&P written QA policy or program for ecotoxicological testing. Nevertheless, QA/QC measures are taken by all EP biotesting labs and they include fish health/holding/acclimation, use of reference toxicants, use of new or improved tests, use of alternative or indigenous test species, and monitoring of the influence of water quality on test results. A great deal of experience has been gained by EP lab personnel for monitoring, legal and program formulation purposes. These staff are encouraged to produce high quality test data both by individual lab managers and by the C&P LMC. Recent work by the Intergovernmental Aquatic Toxicity Group (IGATG) includes the standardization and interlaboratory studies of the acute lethality test in fingerling trout, interlaboratory studies of the acute lethality test with Daphnia magna, and the collection and review of published testing procedures and guidelines. Unfortunately, it cannot be stated that any of the above activities in themselves represent state-of-the-art QA programs; rather, they are efforts toward that end.

Much more extensive and formal QA programs exist, and are continuing to grow, in other countries. For example, the Testing Laboratory Registration Act was enacted in New Zealand in 1972 (OECD, 1982), Good Laboratory Practice (GLP) regulations were promulgated by the U.S. Food and Drug Administration in 1978 (FDA, 1978) and by the U.S. Environmental Protection Agency in 1983 (EPA, 1983), and GLP guidelines were adopted by the OECD in 1981 (OECD, 1982). Several national and international organizations, such as the AOAC, ASTM, ISO, EEC and OECD, also have strong and growing QA programs in place. In this context, it is noteworthy that although Environment Canada has been an active participant in the OECD Chemicals Program and has supported OECD Council decisions and recommendations such as those pertaining to GLP, it has yet to fully implement them in its own laboratories.

Because of the importance placed on GLP by other agencies, it is worthwhile to review this topic briefly. This is provided as Appendix 8A to this report. There is no doubt that the total GLP package as outlined in Appendix 8A would be difficult to accomplish, especially in small laboratories. However, if fully implemented it would give assurance that data produced can be accepted as reliable within known limits of uncertainty.

Given the gap between GLP principles and actual practice in most Canadian testing laboratories, a developmental goal should be to work towards practical adaptation of GLP and related protocols to EP laboratory requirements.

One major attraction of GLP is that it clearly points out that the primary responsibility for the production of reliable test data lies with the individual laboratories, and that it is expected that they are in a position to certify this reliability and to produce documented evidence to this effect. Other attractive features of GLP are its emphasis on the importance of intralaboratory quality control, the need for each laboratory to establish and be able to document performance criteria for each of its procedures (rather than placing blind trust in "standard methods"), the need for corrective procedures, and the need for good and documented work planning. Finally, the existence of a QA program which contains elements of formal GLP should have a positive effect on morale in the laboratory, since such a program would establish management's commitment to the production of reliable data and the importance of each participant in the overall process.

8.3 Major Quality Assurance Elements

The major elements of a suitable QA program for Environmental Protection ecotoxicological testing laboratories are presented and discussed below. It will be noted that these elements are applicable to any laboratory measurement program; specific instances of their relevance to toxicity testing are given as illustrations in the discussion.

Elements of a Quality Assurance Program

A. Quality Assurance Policy
B. Quality Assurance Plans
C. Audit Program
D. Test Protocols
E. QC Procedures
F. Other Standard Operating Procedures
G. Data Certification
H. Corrective Action

Expression of these elements in terms of the components of an ecotoxicological testing laboratory's QA/QC program is provided in Appendix 8B.

Element A: QA Policy

It is important that each level of management in an organization should endorse a QA policy. At the very top, such a policy can be quite straightforward, and can merely state that it is the policy of the organization that there shall be one or more QA programs in place whose purpose is to ensure that laboratory data employed in support of various decision-making activities shall be of assured quality, that adequate funding shall be provided, and that a responsibility centre or centres are identified. At successively lower levels of the organization's structure, expansion and further definition of this policy (so that there is a subsidiary policy statement at each organizational level) serves as the basis for the preparation of increasingly more detailed QA plans.

Element B: QA Plans

As a minimum, QA plans should include the identification of responsibility centres, budget implementation targets for the planning period, and should specify those areas of GLP which are considered to be appropriate at a particular level. (For example, provision for the training of laboratory personnel, for comparable data management systems in all laboratories, for the preparation of standardized audit procedures, sample custody procedures and quality control procedures, although all important at the laboratory level, are probably best planned at the level one above the laboratory, i.e. by the LMC.) Obviously, the greater the input from subordinate levels, the more suitable each plan will be, and the greater will be the commitment of each individual to the overall program. A very detailed example of a QA plan has been prepared for the department's Canadian Air and Precipitation Monitoring Network (Vet and Onlock, 1983), so details will not be repeated here. Rather, it should be sufficient to reiterate that all matters which pertain to the generation of test data (such as sampling, handling, equipment, water quality, test organisms, personnel, facilities, procedures, computation, reporting, record keeping and corrective action) must be addressed in the overall QA plan or plans. The question of laboratory size will be an important consideration in the development of QA plans at the laboratory level, and further deliberation on this point may lead the LMC to formalize QA programs at different rates in different laboratories.

Element C: Audit Program

As has been noted in Appendix 8A the essence of a QA program is that it includes an audit function. At each level of organization, it becomes increasingly important that an independent unit or individual is in a position to certify that the policies, plans, protocols, QC procedures, SOPs, and other important aspects of GLP have been followed, and to notify management through an independent channel if this is not the case. The QA plans at each level must therefore provide for the audit function (which can be as simple as periodic progress reports on the accomplishment of objectives at the most senior levels), AS WELL AS providing for corrective action in the event that the audit identifies discrepancies. In the specific case at hand, the establishment of the audit function, or QA unit, represents a particular challenge in the smaller of the regional laboratories, since the number of functions called for by GLP (audit, management and test performance) equals or exceeds the number of available personnel in these laboratories.

Element D: Test Protocols

Particularly in the cases of "legal" samples and other monitoring for compliance or permit approval purposes, it is important that the test procedures employed can be related to "established", "standard", or "accepted" methods and practices. (This is so because such methods will usually have been intensively studied, compared with other methods, and checked for performance in several different laboratories. In the absence of this sort of documentation of reliability, it becomes incumbent on the testing laboratory to produce some or all of it.) To this end, written protocols or general outlines of each operational test in a given laboratory should be prepared and maintained. Protocols differ from test procedures, in the context of this chapter, in that they specify the scientific basis for the test procedure and enumerate the SOPs employed, the nature and frequency of audits, the QA procedures to be employed, the expected results for known samples, special provisions for sample custody, etc., rather than merely listing the "how to" aspects of a test procedure. Slight modifications of protocols may be desirable or necessary in certain circumstances because of such factors as differing study objectives, number of samples, priorities, scheduling, etc. This eventuality is probably best documented in the form of amendments to a standard protocol for a given test procedure. As previously noted in Chapter 7.0, the IGATG has begun the collection of documentation on the core tests recommended in this report with a view to the establishment of suitable protocols for each of these tests. In the judgement of the IGATG, some effort of this type is required for all of the recommended core tests except the acute lethality test in fingerling trout.

Element E: QC Procedures

Although all SOPs are important, those which are of greatest importance at the inception of a QA program are related to QC procedures: sampling and sample custody; intralaboratory procedures which lead to the setting and checking of tolerances; and the development of criteria for establishing the acceptability of data. QC procedures should embrace every operation which can introduce human error, instrument malfunction, biological variation, or other types of bias.

The procedures which are routinely used for QC purposes should be carefully documented and used by both laboratory staff and the QA unit. As well as indicating exactly how the selected QC procedures should be performed, these documents should indicate why these SOPs are performed, at what frequency they are required, how the data or observations they generate should be reported, and what corrective actions are required when problems are encountered. Alternatively, two sets of QC procedures can be documented; one set for the laboratory staff and one for the QA unit. In this case, the laboratory staff would strictly require only the operational details.

In general terms, QC procedures call for the determination of tolerances for various parameters, for setting reasonable control specifications on the basis of these tolerances, for measuring the tolerances periodically during routine testing, and for taking corrective action when the measured tolerances are found to exceed the control specifications, i.e. when a system is "out of control". The development and use of quality control charts is one widely employed variant of this approach. Quality control charts may be employed to provide evidence of statistical quality control for components of a test procedure, such as the routine calibration of a colorimeter, or for an entire test system, as would be the case for repetitive determination of the median lethal concentration of one or more reference toxicants with a stock test organism. The use of quality control charts is one of the best proven methods for demonstrating that test performance varies only within established tolerances.

Specific QC procedures which should be found among the SOPs for most ecotoxicity testing laboratories include the following:

a) Regular inspection of the health status of test biota in terms of control criteria such as growth rate, morbidity rate, or mortality rate.
b) Proper collection, labelling and preservation of the sample.
c) Real-time checking of all QC sample results against control criteria, so that timely corrective action can be taken.

d) Scheduled checks of important dilution water quality parameters and statistical comparison with previous data.

e) Procedures which are designed to ensure that all recorded measurements and calculations are checked for accuracy and that these checks are audited.

Element F: Other SOPs

All other SOPs are documented procedures which relate to routine operations in the field, the laboratory, and the QA unit. As well as providing concise instructions on exactly how various procedures (sampling, sample handling, weighing, dilution, maintenance, recording, checking, data storage, etc.) are performed, these SOPs also provide the basis for instructing new staff, providing an audit trail of QC records and performance, and for the provision of information on tolerances associated with given equipment, procedures, test organisms, and personnel.

On the basis of the above, it should be clear that these SOPs are, in large part, unique to a given laboratory. The need to develop laboratory-specific SOPs is particularly acute in the area of ecotoxicity because of regional differences in such important variables as water quality, test species, equipment type and condition, and staff expertise. By the same token, however, the sharing of SOPs between laboratories and QA units and their periodic review by all concerned provides a firm basis for the "search for excellence" which is the root of any functioning QA program.

SOPs which should be available for each aquatic toxicity laboratory include the following:

a) Instructions on the acquisition, care, feeding, acclimation, and handling of all test organisms.

b) Details on the treatment, monitoring, control and distribution of dilution water, together with details on required record-keeping.

c) Details on sample handling, storage, pretreatment, sub-sampling, and sample custody.

d) Instructions on preferred methods for carrying out all common laboratory operations, together with details on report forms, instrument logs, maintenance schedules, checking, and record storage.

e) Instructions and worked examples for the recording of measurements and storing of raw data, together with similar illustrations of data handling and the calculation of results.

f) Details on equipment cleaning, checking and maintenance.

g) Documentation on the safe handling of toxic substances, test biota, equipment, and safety procedures in the laboratory.

Element G: Data Certification

Data certification by a responsible laboratory manager is the hallmark of a good QA program. The form of the certificate is open for discussion. At a minimum, however, the certification should state the probable uncertainty associated with the data (this can conveniently be accomplished by means of 95% confidence limits), should summarize the QC methods employed to ensure that the measurement process was in control, should indicate what further information is on file and how it may be obtained, and should list recent results in the same test system with one or more reference toxicants.

Element H: Corrective Action

None of the above elements of a QA program will have the desired effect unless the program also includes clearly defined procedures to trigger for specific corrective action. In other words, a feedback system is essential to a functioning QA program, and may come from the QA unit (audits) or from users of the data as to its relevance/accuracy, etc. Common triggers and initial corrective actions include rejection of data generated during times at which the test method is known or suspected to be out of statistical control, rejection of animal test data if control mortality or morbidity exceeds previously-established tolerances, provision for a laboratory manager to refuse to accept samples which have not been collected, identified, preserved, transported or handled in accord with previously-established SOPs, etc. Specific follow-up corrective measures/actions will then be required to ensure problems are rectified and subsequent data are of suitable quality.

Authority for the most drastic corrective actions is normally vested in the project manager and in the QA unit, and good QA plans will define the limits of authority for all specified corrective actions. Failure to establish data acceptability criteria and to provide for remedial action appears to be one of the most common oversights in newly-established QA programs.

8.4 Costs of Quality Assurance

Although actual studies of the cost of establishing a QA program are very few and far between, there does appear to be a consensus that the minimum cost which should be seriously entertained is of the order of 10 - 15% of the analytical costs. This figure applies to the operating costs of an established program; in the context of this document, start-up costs are perhaps of greater interest. Here, it can be noted that even the smallest laboratory can afford to provide a small amount of time to putting pieces of a QA program in place; that the costs lie completely within the control of the laboratory managers; that start-up costs are predominantly O&M and salary in nature; and that, since QA costs are largely independent of the number of tests performed, the most cost-effective way to introduce a QA program is to start with the most frequently-performed operations in a given laboratory.

8.5 Quality Assurance and Contracting-Out

The department would be pressed, on occasion, if required to establish that its toxicity testing data obtained under contract were fully supported by the kinds of considerations discussed in Chapter 8.3. Given the care, collaboration, and time required to establish a functioning QA program within departmental laboratories, it should be no surprise that ensuring that parallel QA programs exist in contract laboratories represents a comparatively difficult problem.

There appear to be only three possible approaches to assuring the quality of toxicity testing data which have been contracted-out. These are: a) to certify private laboratories for specific testing; b) to assume that extramural laboratories are competent and produce data of "good quality"; or c) to require the laboratories to meet certain standards of performance prior to the award of contracts and to provide evidence of statistical quality control during the progress of the work. As was noted in Chapter 8.2, approach a) is favoured in most Western countries, and could also become the preferred approach in Canada, should the longstanding NH&W proposal to Cabinet on GLP ever be approved. The State of New Jersey has begun such a program for bioassay laboratories (N.J. Dept. of Env. Protection, 1984). The drawback of this approach is that it requires considerable resources to implement, which are not available to C&P at present. Approach b) is clearly unacceptable in even a minimal QA program. Approach c) might be described as a negotiated self-certification by the private laboratories, or as a poor man's certification program. This approach has been chosen, initially for certain chemical analyses in sediments, by the Ocean Dumping Program, and appears to have the merit that contractors who have been involved with preliminary trials of the system approve of it. This approach is also similar to that which was recommended to the Toxic Chemicals Steering Committee a few years ago (Norstrom et al. 1980) and to that which is employed by CWS for certain analytical chemistry which is contracted-out.

In order for approach c) to be put in place, it would be necessary to have a very detailed understanding of operations, equipment, personnel, etc. in the contracting laboratories, to obtain agreement with DSS and contracting laboratories that proficiency tests are a prerequisite to the awarding of a contract, to obtain real-time results for all check and quality control samples analyzed (under "blind" conditions) by the laboratories, and to ensure that appropriate performance and penalty clauses were added to testing contracts. Furthermore, training of departmental scientific authorities and auditors would also be necessary before ecotoxicological testing could be performed extramurally on a broad scale under conditions of assured quality. Program managers will have to plan studies in advance to allow consultant labs adequate time to institute proper QA/QC measures. In this regard, the practice of using year-end money for contract work has caused problems in the past and should be discouraged where possible.

To make the above possible, the EP biotesting labs (or the IGATG) could be given an advisory role to ensure that all contract proposals:

i) Are properly designed to meet the study objectives
ii) Utilize the proper test methods
iii) Include a QA/QC program.

8.6 RECOMMENDATIONS

(a) Immediate/Short-Term

1. The LMC should prepare and endorse a QA plan for the conduct of ecotoxicological tests in and for Environmental Protection laboratories.

2. Each laboratory manager should prepare and endorse a detailed QA plan for his laboratory. These plans should be based on the LMC QA plan, and should include specific objectives, responsibilities, etc.

3. The LMC should continue development of QA plans for any contracted-out work which involves the core tests recommended in this report.

4. The LMC should initiate the preparation of draft SOPs for all procedures which are relevant to ecotoxicological testing. These should include, but not be restricted to: sampling; sample custody; the use of split, spiked, and blind replicate samples; methods for statistical control of repetitive operations; procurement and care of test organisms; criteria for the acceptability of test data (control mortality and morbidity, acceptable limits for controlled parameters, etc.); standardized methods of record-keeping, performing calculations and reporting of numerical data; together with SOPs for the checking and auditing of these and related functions.

5. The individual laboratory managers, after appropriate consultation, should initiate the preparation and review of SOPs for all procedures which are unique to their laboratories. Initial emphasis should be placed on those laboratory operations involved in the core tests specified in this report.

(b) Long-Term

6. The LMC should prepare and endorse a QA policy with regard to the generation of ecotoxicological data by or for Environmental Protection laboratories and/or programs. The Committee should also advise the C&P Management Board of the advisability of preparing and endorsing appropriate QA policies for all laboratory data produced for or by C&P with initial emphasis on those data employed for regulatory, monitoring, and policy formulation purposes.

7. The LMC should consider the establishment of a formal training program on QA and QC. Such a program could be begun by holding a combined conference and workshop, at which some of the preparation and review of policies, plans, and SOPs noted above could be accomplished.

9.0 METHODOLOGY RESEARCH AND DEVELOPMENT

(Prepared by C. Blaise, R. Parker and G. Sergy)

9.1 Identification of Needs

The state-of-the-art in biological testing is continually evolving, as it should be, taking advantage of new knowledge to improve the tools now available and develop those we require. There are many R&D needs which must be satisfied so that relevant biotests can be applied to more effectively evaluate dangers to the environment. Major areas where such efforts should be directed are identified below.

1. Core test methods development and standardization
2. Biotest development related to specialized regional needs
3. Laboratory and field biotesting linkages
4. Legal concerns associated with biotests
5. Hazard assessment scheme (HAS) development
6. Practical Cost-Efficient (PCE) tests development and/or application
7. Development/application of biotests related to health effects
8. Development/application of biotests to assess impact on the terrestrial environment.

Of these eight categories of developmental requirements the highest priority, from the perspective of the Lab Managers Committee (LMC), is attached to work on the core tests (1). Specific suggestions under all others need to be assessed on an individual basis to meet the most pressing demands in line with current C&P strategies.

1. **Core Test Methods Development and Standardization**

Chapter 4.0 outlines the selection of core aquatic toxicity tests for EP. Many of these tests do require some additional effort before a national protocol is adopted. This includes such items as the evaluation and selection of the most suitable species (e.g. for amphipod sediment assay) or existing protocol(s) (e.g. for daphnid life cycle test) or test types (e.g. for genotoxicity). It may require slight modifications or consolidation of existing protocols to better reflect Canadian conditions and problems. These items are detailed in Chapter 7.0. While such activities are not research per se, they are the fine-tuning and completion stage of methods development and should be afforded the highest priority for EP labs.

In addition, developmental activities related to expanding the application and versatility of the core tests protocols, instrumentation and test species should be given a high profile. It is obviously practical to increase the number of end-points for species which are maintained and to increase the number of uses for any particular test or equipment.

2. Biotest Development Related to Specialized Needs

The different geographic and administrative regions in Canada have different environmental problems and needs. Chapter 4.3 provided a listing of specialized regional needs as identified by regional managers. Many of those require further methods or protocol development. Many of those tests, once ready, would also have wider applicability to EP programs.

3. Laboratory and Field Biotesting Linkages

There is a need to show adequate compatibility between laboratory- and field-generated biotesting results. This is especially true in view of the 'toxics strategy' which we are adopting. Several avenues of R&D are required in an attempt to harmonize laboratory and field techniques.

- Develop/apply biotests reflecting results from community trend analysis, those which could act as surrogates to traditional impact monitoring techniques.
- Develop programs to improve relationships between toxicity tests and receiving water effects (compare effects monitoring with end of pipe biotesting).
- Verify response relationships between standard test species and indigenous resource species.

- Adapt/modify laboratory procedures to better reflect field conditions.
- Assess and promote cost-efficiency of field biomonitoring methods.

4. Legal Concerns Associated with Biotests

Although this area is not an R&D endeavour per se, it certainly is very much intertwined with biotesting and much is left to accomplish in order to resolve legal matters related to biotest results. Efforts in this sphere of activity should include:

- improving the acceptability and legal ease in using information other than the present regulatory test (e.g. using surrogate testing at the same or different trophic and/or end-point levels);
- training of legal expert witnesses;

5. Hazard Assessment Scheme (HAS) Development

HAS strategies are useful in identifying and characterizing the ecotoxicity problems inherent to complex waste samples, for priorizing future corrective actions and making decisions on specific control measures. HASs are an integrated ecotoxicological tool which require fine tuning and refining by those who employ them. There is a need

- to update test batteries periodically to ensure the best sensitivity and cost-effectiveness: this implies staying abreast of new R&D in ecotoxicology and modifying HASs accordingly;
- to demonstrate the validity and usefulness of HASs and verify its utility for decision-making;
- to evaluate and "standardize" HASs by undertaking interlaboratory comparisons to assess their precision and reproducibility;
- to apply/develop causally-related biotests such that a noted effect can be attributed to a particular class of aggression. Biotechnology may be able to construct bioengineered micro-organisms that are sensitive to particular classes of chemicals. Such tests will contribute to augment cost-efficiency of HASs by avoiding unnecessary effort and costs associated with "blind scanning" of chemicals performed in an attempt to explain noted effects.

6. Practical Cost-Efficient (PCE) Tests

There are numerous reasons for developing and applying PCE tests in Environmental Protection Toxicology Laboratories. Simplicity, rapidity, cost-effectiveness and low-volume requirement are some of these. It is desirable, furthermore, for these tests to be as sensitive and representative as possible. Such tests are also attractive because, quite often, they can lend themselves to miniaturization and automation. This can further decrease labour-intensiveness and therefore increase cost-efficiency. Examples of R&D needs for PCE tests are outlined below:

- use of PCE tests for both laboratory and field situations;
- assess screening potential and PCE features of microbial tests other than Microtox (where reagent purchase is exclusive to one firm);
- continue to assess new PCE biotests: investigate their sensitivity, predictive capabilities and cost-effectiveness;
- develop/apply a PCE test at the protozoan level (acute lethal and acute sublethal) as part of an aquatic assessment battery;
- undertake PCE test - fish test comparative studies in an attempt to find a useful "equivalency test" for the trout bioassay for both laboratory and field applications.

7. Tests Related to Health Effects on Aquatic Biota and/or Humans

Toxicity, genotoxicity, bioaccumulation and biodegradation can be reflective of potential health effects on aquatic organisms, and, in some cases, humans. There are pressing needs for R&D in these sectors.

Toxicity: Biotests are required to appraise "state of health", given low-level long-term exposures to contaminants.

Genotoxicity: More health-related tests are necessary to assess danger to both aquatic organisms and humans with regard to industrial discharges. Predictive value and data interpretation of such tests must be improved. - There is a need to assess various types of rapid screening tests (Ames, Chromotest, dark mutant, etc.) with regard to complex waste appraisals. Investigations which apply a tiered approach to better predict the danger and associated risk of genotoxicants present in wastewater discharges are required. Appraisal/application of fish genotoxicity tests are also necessary (e.g. fathead minnow teratogenicity test).

Bioaccumulation: Rapid screening types of biological tests to initially identify potential bioaccumulation problems linked to effluents are required (e.g. inorganic, organic and genotoxic bioaccumulation potential in algae).

Biodegradation: The development/application of a standardized test to assess the active (with seed) and passive (without seed) biodegradation of effluent toxicity components is required. An appraisal of OECD "standardized tests" to assess biodegradability of priority chemicals and effluents should be conducted. Knowledge on fate and effects of contaminants in this respect is essential.

Biotechnology: Tests are required to investigate the impact of "biotechnology waste streams" and subsequent release of bioengineered micro-organisms to receiving waters.

8. Development/Application of Biotests to Assess Impact on the Terrestrial Environment

Land disposal of wastes is a growing practice which must be assessed via biotests as is atmospheric fallout of pollutants on sensitive terrestrial environments. EP activity in this area is expected to increase in the future. There is an urgent need to develop/apply a battery of tests to assess ecotoxicity impacts on land. As in the case of water-based aggressions, a genuine hazard assessment strategy is a desirable approach. Biotests involved in this type of evaluation should include earthworm, seed inhibition germination, Microtox, lichens, and plant genotoxicity assays as well as others employed in wildlife toxicology.

9.2 Options for Conducting Research and Developmental Work

In-House

Within C&P, research and development on aquatic biological tests have been conducted in both EP and IWD facilities. The latter, directed from NWRI Burlington, has a clear mandate for such activities. In EP laboratories R&D has been a restricted activity, often limited to simple refinements of test methods and aspects of quality control. Probably due to the efforts of individual scientists and managers, certain regional laboratories have conducted much more R&D than others. In some cases R&D is given more legitimacy such as in support of regulations development or of other internal branches which have a 'valid' R&D mandate, e.g. TDTSB. Generally, however, there is an attitude which discourages R&D in EP labs and relegates it to a closet activity. Lab resources spent on R&D have been viewed as subtracting from those resources available to support 'real' programs. Often in-house work has been conducted only when time away from regular laboratory analysis was available.

In-house R&D has many positive features. Knowledgeable staff of scientists, biologists and technicians exist to carry out R&D work if authority and resources were available. In some cases the facilities and/or exertise may not exist privately. EP laboratory personnel work in close association with the program staff, which provides them with a much better insight into the problems being addressed. Most R&D work identified in the foregoing document relates to methods development, improvements, standardization and testing. EP laboratory people are all experienced in these areas. Additionally, there is the benefit of training and maintaining staff necessary to provide vital knowledge and expertise to many associated C&P activities.

Variants of the in-house option which have or could be successfully utilized include the use of co-op students; MSc.-, Ph.D.-, post-doctorate candidates; in-house contractors (see below); shared facilities or joint programs with other agencies. All of these tend to favour the knowledge-gain-to-resource-use ratio.

While in-house applied research using available staff and facilities is a minor expense item, it can be slow in achieving results if the activity is not recognized as a 'real' work item.

Contract Out

The second option for conducting R&D work is contracting to the private sector (consultants, universities, industrial research centres). There are many very well qualified researchers available in the private sector who could carry out the R&D work recommended in this document. Generally, contract research is costly but the product is delivered on a very timely basis. With properly controlled contracting out procedures (terms of reference/statements of work, requests for proposals, qualified project management committee, quality assurance and strict control over finances and timing) a quality product can certainly be delivered.

A combination of 1 and 2 has been used in many instances in the past. Research funds have been made available to the EP laboratories to contract out specific projects which are usually conducted within EP facilities using EP equipment. The work can be closely directed by the EP staff and because the contract is dedicated to a particular project, the work is completed on a timely basis. Since government facilities are used, the cost of these contracts is much less than using laboratories in the private sector.

UPs

The use of unsolicited proposals through DSS has been a very cost-efficient route for obtaining R&D work. Unfortunately, by the nature of the program, there is less direct control over the objectives of the proposal or the methods used to obtain those objectives. Certainly though, with good contract management and advice from the Department, some types of R&D needs can be met with a quality product and on a timely basis.

9.3 Recommendations

1. It is recommended that research and development needs in the area of biological testing be met through a balanced approach utilizing all of the options described above.

2. EP in-house research activities should be recognized as a legitimate and necessary function and for the benefit it provides. The LMC should indicate, on a national scale, a level of in-house effort to meet projected needs.

3. R&D on aquatic toxicity testing in C&P would likely be more effective through an alignment of IWD and EP objectives and subsequent cooperative efforts.

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Appendix 1

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Appendix 1A.

Policy for the Development of Water Quality-based Permit Limitations for Toxic Pollutants

(taken from ref. EPA 1984a)

SUPPLEMENTARY INFORMATION: As the water pollution control effort in the United States progresses and the "traditional" pollutants (oxygen demanding and eutrophying materials) become sufficiently treated to protect water quality, attention is shifting towards pollutants that impact water quality through toxic effects. Compared with the traditional pollutants, regulation of toxic pollutants is considerably more difficult. The difficulties include (1) the great number of toxic chemicals that may potentially be discharged to receiving waters and the difficulties in their analysis; (2) the changes in the toxic effects of a chemical resulting from reactions with the matrix of constituents in which it exists; and (3) the inability to predict the effects of exposure to combinations of chemicals.

To overcome some of these problems, EPA and the States have begun to use aquatic toxicity tests and various human health assessment techniques to complement chemical analyses of effluents and receiving water samples. Because these techniques or their application to effluent testing are new, EPA and the States have been cautious in their use. Based on EPA's evaluation of these techniques and the experiences of several States, EPA is now recommeding the use of biological techniques as a complement to chemical-specific analyses to assess effluent discharges and express permit limitations. EPA has issued these recommendations through a statement of policy and is developing a technical guidance document to help implement the policy.

The complete test of the national policy statement follows:

Policy for the Development of Water Quality-Based Permit Limitations for Toxic Pollutants

Statement of policy

To control pollutants beyond Best Available Technology Economically Achievable (BAT), secondary treatment, and other Clean Water Act technology-based requirements in order to meet water quality standards, the Environmental Protection Agency (EPA) will use an integrated strategy consisting of both biological and chemical methods to address toxic and nonconventional pollutants from industrial and municipal sources. Where State standards contain numerical criteria for toxic pollutants, National Pollutant Discharge Elimination System (NPDES) permits will contain limits as necessary to assure compliance with these standards. In addition to enforcing specific numerical criteria, EPA and the States will use biological techniques and available data on chemical effects to assess toxicity impacts and human health hazards based on the general standard of "no toxic materials in toxic amounts."

EPA, in its oversight role, will work with States to ensure that these techniques are used wherever appropriate. Under section 308 and section 402 of the Clean Water Act (the Act), EPA or the State may require NPDES permit applicants to provide chemical, toxicity, and instream biological data necessary to assure compliance with standards. Data requirements may be determined on a case-by-case basis in consultation with the State and the discharger.

Where violations of water quality standards are identified or projected, the State will be expected to develop water quality-based effluent limits for inclusion in any issued permit. Where necessary, EPA will develop these limits in consultation with the State. Where there is a significant likelihood of toxic effects to biota in the receiving water, EPA and the States may impose permit limits on effluent toxicity and may require an NPDES permittee to conduct a toxicity reduction evaluation. Where toxic effects are present but there is a significant likelihood that compliance with technology-based requirements will sufficiently mitigate the effects, EPA and the States may require chemical and toxicity testing after installation of treatment and may reopen the permit to incorporate additional limitations if needed to meet water quality standards. (Toxicity data, which are considered "new information" in accordance with 40 CFR 122.62(a)(2), could constitute cause for permit modification where necessary.)

To carry out this policy, EPA Regional Administrators will assure that each Region has the capability to conduct water quality assessments using both biological and chemical methods and provide technical assistance to the States.

Background

The Clean Water Act establishes two principal bases for effluent limitations. First, existing dischargers are required to meet technology-based effluent limitations that reflect the best controls available considering economic impacts. New source dischargers must meet the best demonstrated technology-based controls. Second, where necessary, additional requirements are imposed to assure attainment and maintenance of water quality standards established by the States and approved by EPA. In establishing or reviewing NPDES permit limits, EPA must ensure that the limits will result in the attainment of water quality standards and protect designated water uses, including an adequate margin of safety.

For toxic and nonconventional pollutants it may be difficult in some situations to determine attainment or nonattainment of water quality standards and set appropriate limits because of complex chemical interactions which affect the fate and ultimate impact of toxic substances in the receiving water. In many cases, all potentially toxic pollutants cannot be identified by chemical methods. In such situations, it is more feasible to examine the whole effluent toxicity and instream impacts using biological methods rather than attempt to identify all toxic pollutants, determine the effects of each pollutant individually, and then attempt to assess their collective effect.

The scientific basis for using biological techniques has advanced significantly in recent years. There is now a general consensus that an evaluation of effluent toxicity, when adequately related to instream conditions, can provide a valid indication of receiving system impacts. This information can be useful in developing regulatory requirements to protect aquatic life, especially when data from toxicity testing are analyzed in conjunction with chemical and ecological data. Generic human health effects methods, such as the Ames mutagenicity test, and structure-activity relationship techniques are showing promise and should be used to identify potential hazards. However, pollutant-specific techniques are the best way to evaluate and control human health hazards at this time.

Biological testing of effluents is an important aspect of the water quality-based approach for controlling toxic pollutants. Effluent toxicity data in conjunction with other data can be used to establish control priorities, assess compliance with State water quality standards, and set permit limitations to achieve those standards. All States have water quality standards which include narrative statements prohibiting the discharge of toxic materials in toxic amounts. A few State standards have criteria more specific than narrative criteria (for example, numerical criteria for specific toxic pollutants or a toxicity criterion to achieve designated uses). In States where numerical criteria are not specified, a judgment by the regulatory authority is required to set quantitative water quality-based limits on chemicals and effluent toxicity to assure ompliance with water quality andards.

Note.—Section 308 of the Act and corresponding State statutes authorize EPA and the States to require of the owner/operator any information reasonably required to determine permit limits and to determine compliance with standards or permit limits. Biological methods are specifically mentioned. Toxicity permit limits are authorized under Section 301 and 402 and supported by Section 101.

Application

This policy applies to EPA and the States. The policy addresses the use of chemical and biological methods for assuring that effluent discharges are regulated in accordance with Federal and State requirements. This policy was prepared, in part, in response to concerns raised by litigants to the Consolidated Permit Regulations (see FR 52079, November 18, 1982). Use of these methods for developing water quality standards and trend monitoring are discussed elsewhere (see 48 FR 51400, November 8, 1983 and *Basic Water Monitoring Program* EPA–440/9–76–025). This policy is part of EPA's water quality-based control program and does not supersede other regulations, policy, and guidance regarding use tainability, site-specific criteria modification, wasteload allocation, and water quality management.

Implementation

State Role

The control of toxic substances to protect water quality must be done in the context of the Federal-State partnership. EPA will work cooperatively with the States in identifying potential water quality standards violations, assembling relevant data, developing appropriate testing requirements, determining whether standards are being violated, and defining appropriate permit limits.

Note.—Under sections 303 and 401 of the Act, States are given primary responsibility for developing water quality standards and limits to meet those standards. EPA's role is to review the State standards and limits and develop revised or additional standards or limits as needed to meet the requirements of the Act.

Integration of Approaches

The type of testing that is most appropriate for assessing water quality impacts depends on the type of effluent and discharge situation. EPA commends that an integrated pproach, including both biological and chemical techniques, be used to assess and control water quality. The principal advantages of chemical-specific techniques are that (1) chemical analyses are usually less expensive than biological measurements in simple cases; (2) treatment systems are more easily designed to meet chemical requirements than toxicity requirements; and (3) human health hazards and bioaccumulative pollutants can best be addressed at this time by chemical-specific analysis. The principal advantages of biological techniques are that (1) the effects of complex discharges of many known and unknown constituents can be measured only by biological analyses; (2) bioavailability of pollutants after discharge is best measured by toxicity testing; and (3) pollutants for which there are inadequate chemical analytical methods or criteria can be addressed.

Pollutant-specific chemical analysis techniques should be used where discharges contain few, well-quantified pollutants and the interactions and effects of the pollutants are known. In addition, pollutant-specific techniques should be used where health hazards are a concern or bioaccumulation is suspected. Biological techniques should be used where effluents are complex or where the combined effects of multiple discharges are of concern. EPA recognizes that in many cases both types of analysis must be used.

Testing Requirements

Requirements for dischargers to collect information to assess attainment or nonattainment of State water quality standards will be imposed only in selected cases where the potential for nonattainment of water quality standards exists. Where water quality problems are suspected but there is a strong indication that complying with BCT/BAT will sufficiently mitigate the impacts, EPA recommends that applicable permits include testing requirements effective after BCT/BAT compliance and reopener clauses allowing reevaluation of the discharge.

The chemical, physical, and biological testing to be conducted by individual dischargers should be determined on a case-by-case basis. In making this determination, many factors must be considered, including the degree of impact, the complexity and variability of the discharge, the water body type and hydrology, the potential for human health impact, the amount of existing data, the level of certainty desired in the water quality assessment, other sources of pollutants, and the ecology of the receiving water. The specific data needed to measure the effect that a discharger has on the receiving water will vary according to these and other factors.

An assessment of water quality should, to the extent practicable, include other point and nonpoint sources of pollutants if the sources may be contributing to the impacts. Special attention should be focused on Publicly Owned Treatment Works (POTW's) with a significant contribution of industrial waste-water. Recent studies have indicated that such POTW's are often significant sources of toxic materials. When developing monitoring requirements, interpreting data, and determining limitations, permit engineers should work closely with water quality staff at both the State and Federal levels.

A discharger may be required to provide data upon request under section 308 of the Act, or such a requirement may be included in its NPDES permit. The development of a final assessment may require several iterations of data collection. Where potential problems are identified, EPA or the State may require monitoring to determine whether more information is needed concerning water quality effects.

Use of Data

Chemical, physical, and biological data will be used to determine whether, after compliance with BCT/BAT requirements, there will be violations of State water quality standards resulting from the discharge(s). The narrative prohibition of toxic materials in toxic amounts contained in all State standards is the basis for this determination taking into account the designated use for the receiving water. For example, discharges to waters classified for propagation of cold water fish should be evaluated in relation to acute and chronic effects on cold water organisms, potential spawning areas, and effluent dispersion.

Setting Permit Limitations

Where violations of water quality standards exist or are projected, the State and EPA will determine pollution control requirements that will attain the receiving water designated use. Where effluent toxicity is an appropriate control parameter, permit limits on effluent toxicity should be developed. In such cases, EPA may also require a permittee to conduct a toxicity reduction evaluation. A toxicity reduction evaluation is an investigation conducted within a plant or municipal system to isolate the sources of effluent toxicity, specific causative pollutants if possible, and determine the effectiveness of pollution control options in reducing the effluent toxicity. If specific chemicals are identified as the cause of the water quality standards violation, these individual pollutants should be limited. If a toxicity reduction evaluation demonstrates that limiting an indicator parameter will ensure attainment of the water quality-based effluent toxicity requirement, limits on the indicator parameter should be considered in lieu of limits on effluent toxicity. Such indicator limits are not limits on causative pollutants but limits demonstrated to result in a specific toxicity reduction.

Monitoring

Where pollution control requirements are expressed in terms of a chemical or toxicological parameter, compliance monitoring must include monitoring for that parameter. If an indicator parameter is used based on the results of a toxicity reduction evaluation, periodic toxicity testing may be required to confirm the adequacy of the indicator. Where biological data were used to develop a water quality assessment or where the potential for water quality standards violations exist, biological monitoring (including instream monitoring) may be required to ensure continuing compliance with water quality standards.

EPA believes that the intelligent application of an integrated strategy using both biological and chemical techniques for water quality assessment will facilitate the development of appropriate controls and the attainment of water quality standards. EPA looks forward to working with the States in a spirit of cooperation to further refine these techniques.

Policy signed February 3, 1984 by Jack E. Ravan, Assistant administrator for Water.

Dated: February 16, 1984.

Jack E. Ravan,

Assistant Administrator for Water.

[FR Doc. 84-6445 Filed 3-8-84; 8:45 am]

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Appendix 1B. Approaches to Water Quality-based Toxics Control *

The Whole-effluent Approach

The whole-effluent, or toxicity-based, approach to toxics control involves the use of toxicity tests to measure the toxicity of industrial and municipal wastewater discharges. A toxicity test is a relatively simple laboratory procedure. An effluent sample is collected and diluted in test chambers; usually the dilutions used are 100%, 30%, 10%, 3%, 1%, and a control. Often the receiving water is used to dilute the effluent because it more closely simulates effluent/receiving water interactions. Test organisms are placed in these test chambers for specified periods of time. At various points during the testing period, the number of organisms affected in each chamber is counted (the endpoint can be mortality, lower fecundity, reduced growth rates, etc.) and the lowest effluent concentration that causes that endpoint is calculated. This endpoint concentration becomes a quantified measure of the concentration that would cause instream impact if exceeded for a particular period of time. It is usually stated either as an LC50 (the effluent concentration at which 50% of the test organisms are killed) or a No Observed Effect Level or NOEL (the highest effluent concentration at which no unacceptable effect will occur even at continuous exposure).

The measurement of whole-effluent toxicity can then be used to limit the discharge of toxicants in an effluent. Toxicity itself is used as the effluent parameter. The toxicants creating that toxicity need not be specifically identified or controlled where the effluent's toxicity is limited. An analogy between effluent toxicity and biochemical oxygen demand (BOD) can be drawn. Both are measurements of a biological effect. Both can be quantified. In neither case are the causative agents of the biological effect specifically identified. Thus, whole-effluent toxicity is like BOD in that it is a useful parameter for reducing an undesirable effect caused by the discharge of a complex mixture of waste materials.

The Chemical-specific Approach

The chemical-specific approach to toxics control involves the use of laboratory-generated water quality criteria or State standards to limit specific toxicants directly. The toxicity analysis of those chemicals is done in a comprehensive testing program that, unlike whole-effluent testing, attempts to consider a range of toxic endpoints including human health impact and bioaccumulation. Once a criterion is developed, the number is applied, through an exposure analysis, as a permit limit to ensure that the level of that toxicant is not exceeded after discharge. Fate modeling of the toxicant to estimate its behavior after discharge can be an important step in establishing water quality-based permit limits.

Advantages and Disadvantages of the Approaches

The principal advantages of chemical-specific techniques are:

- Treatment systems are more easily designed to meet chemical requirements because more treatability data are available and treatment engineers and permit writers are more familiar with the procedures.
- The fate of a pollutant can be measured through modeling.
- Chemical analyses, in simple cases, can be less expensive than toxicity testing.
- Specific problem chemicals such as carcinogens or bioaccumulative chemicals can be directly limited.

The principal disadvantages of chemical-specific techniques are:

- All toxicants in complex wastewaters may not be known and, therefore, control requirements for all potential toxicants cannot be set.
- It is not always clear which is the toxic compound(s) in the mixture.
- It can become expensive to measure individual toxicants, particularly where many are present in the mixture. Organic chemicals can, in particular, be costly to measure.
- The bioavailability of the toxicants at the discharge site are not assessed, and the interactions between toxicants (e.g., additivity, antagonism) are not measured or accounted for.

The principal advantages of the whole-effluent approach are:

- The aggregate toxicity of all constituents in a complex effluent is measured, and toxic effect can be limited by limiting one parameter — effluent toxicity.
- The bioavailability of the toxic constituents is assessed, and the effects of interactions of constituents are measured.

The principal disadvantages of the whole-effluent approach are:

- Effluent toxicity treatability data are lacking, and treatment engineers and permit writers are unfamiliar with the procedures.
- Where there are chemical/physical conditions (pH changes, photolysis, etc.) present that act on toxicants in such a way as to "release" toxicity downstream, such toxicity may not be measured in the effluent.
- Properties of specific chemicals in complex effluents (such as bioaccumulation, carcinogenicity) are not assessed.

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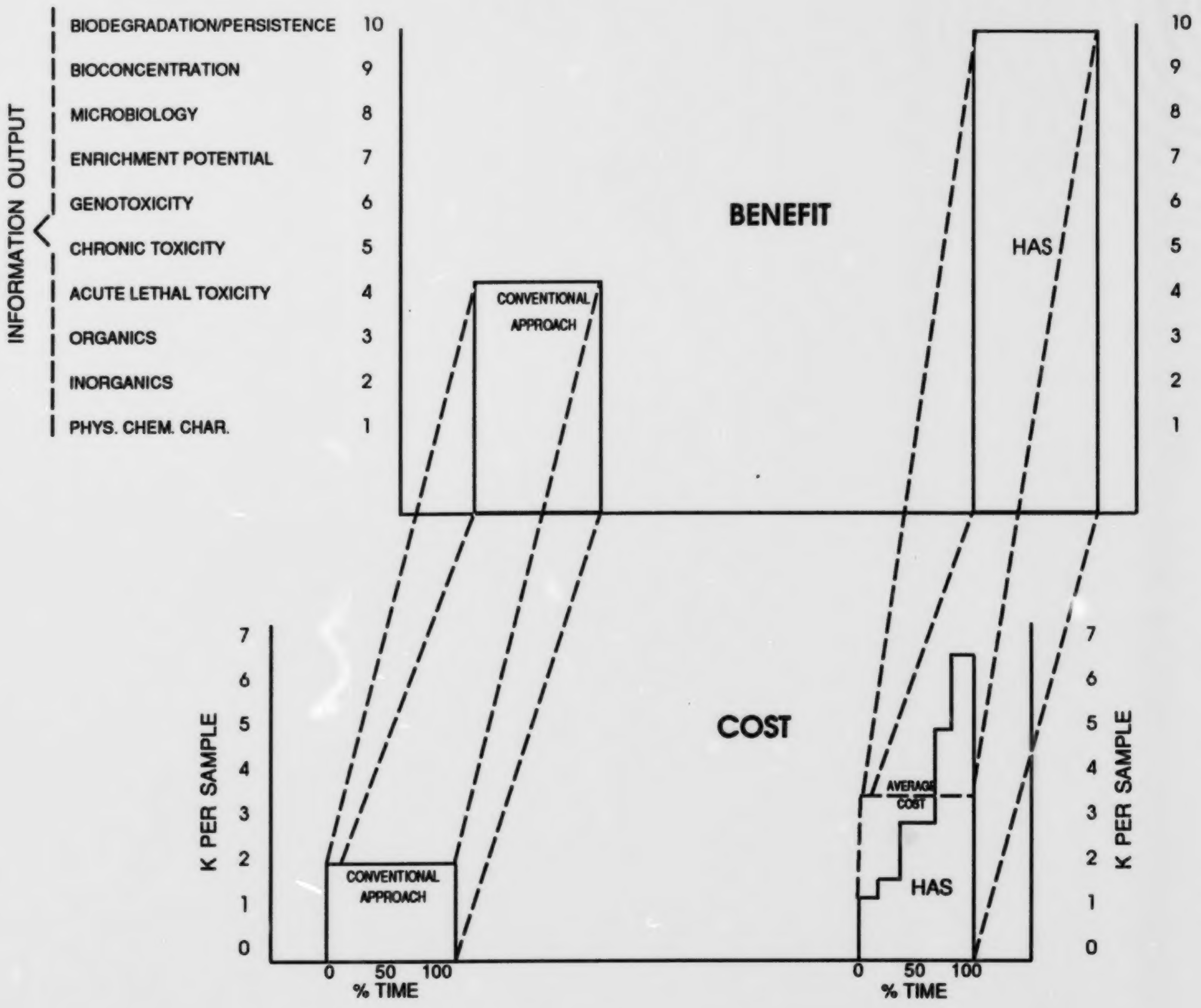
* taken from EPA 1985a. Technical Support Document for
Water Quality-based Toxics Control.

Appendix 2

Appendix 2A.	A Cost/Benefit Comparison Between Conventional and HAS Approaches
Appendix 2B.	Biotests Potentially Useful in Relating Observed Effects to Causally-related Organic or Inorganic Toxic Aggression
Appendix 2C.	Issues on the Use of Aquatic Toxicity Tests


INFORMATION OUTPUT
BIODEGRADATION/PERSISTENCE 10
BIOCONCENTRATION 9
MICROBIOLOGY 8
ENRICHMENT POTENTIAL 7
GENOTOXICITY 6
CHRONIC TOXICITY 5
ACUTE LETHAL TOXICITY 4
ORGANICS 3
INORGANICS 2
PHYS. CHEM. CHAR. 1
BENEFIT
CONVENTIONAL APPROACH
HAS
10
9
8
7
6
5
4
3
2
1
COST
K PER SAMPLE
7
6
5
4
3
2
1
0
CONVENTIONAL APPROACH
0
50
100
% TIME
AVERAGE COST
HAS
0
50
100
% TIME
K PER SAMPLE

Appendix 2A. A cost/benefit comparison between conventional and HAS approaches.
(Blais et al 1987)

Appendix 2B. Biotests Potentially Useful in Relating Observed Effects to Causally-related Organic or Inorganic Toxic Aggression. (Blaise et al. 1987).

<table>
<tr><th>Biotest Procedures</th><th>Reference</th><th>Interpretation potential</th></tr>
<tr><td>1. <i>P. phosphoreum</i> Microtox assay
and
<i>S. Capricornutum</i> algal microtest</td><td>Beckamn, 1982

Blaise <i>et al.</i>, 1986a</td><td>Since the Microtox and algal assays are generally more sensitive to organic and inoganic toxicants, respectively, test results can help to predict the nature of aggression, as shown below:
<table>
<tr><th colspan="2">Test result possibilities</th><th>Probable nature of aggression</th></tr>
<tr><th>Microtox</th><th>Algal microtest</th><th></th></tr>
<tr><td>+</td><td>+</td><td>Inorganic and possibly organic</td></tr>
<tr><td>+</td><td>-</td><td>Organic</td></tr>
<tr><td>-</td><td>+</td><td>Inorganic</td></tr>
</table></td></tr>
<tr><td>2. Algal/Microtox combined assay to assess organic bioconcentration potential of effluents</td><td>Blaise <i>et al.</i>, 1981</td><td>After exposure of algae to effluents, cells are collected, lysed and assayed with Microtox: lysate toxicity denotes toxic accumulation of an organic nature.</td></tr>
<tr><td>3. Algal/algal combined assay to assess inorganic bioconcentration potential of effluents</td><td>Unpublished results</td><td>After exposure of algae to effluents, cells are collected, irradiated (U.V.) to destroy organic content, and assayed with the algal microtest: resulting toxicity denotes toxic accumulation of an inorganic nature.</td></tr>
<tr><td>4. Inorganic toxicity diplayed by Na_2EDTA addition in algal toxicity bioassays</td><td>Couture <i>et al.</i>, 1985</td><td>Na_2EDTA chelates inorganic toxicants and consequently modifies toxic effects. When an algal biomass (B) measured from EDTA-enriched samples is compared to an algal biomass obtained without enrichment, a value of $B_{EDTA}/B \geq 1.3$ indicates the presence of toxic inorganic substances.</td></tr>
</table>

Appendix 2C. Issues on the Use of Aquatic Toxicity Tests.

Correlation of Effluent Toxicity Measurements to Actual Instream Impact.

It is generally agreed that further studies are needed on field validation, i.e. the validity of lab toxicity tests in predicting instream toxicity and ecosystem degradation. Nevertheless, "EPA's Complex Effluent Toxicity Testing Program has shown that at each of the eight sites investigated to date (Lima, Ohio; Circleville, Ohio; Birmingham, Alabama; Baltimore, Maryland; Waterbury, Connecticut; Enid, Oklahoma; Charleston, West Virginia; and Wheeling, West Virginia), when exposure and potential chemical interactions are assessed, effluent toxicity can be correlated directly to impact in receiving waters. This relationship is proving valid in both unimpacted sites (Circleville) and impacted sites (the other sites listed above)." (EPA 1985a).

EPA is thus satisfied that an evaluation of effluent toxicity, when adequately related to instream conditions, can give a valid assessment of receiving system impact. This is also true in the application of chemical-specific water quality criteria for toxicants. Provided exposure is adequately assessed, measured instream concentrations of individual chemicals exceeding established safe concentrations can be considered to cause unacceptable impact to the receiving system.

Variability within Toxicity Test Methods.

Research into this issue by various groups has shown that toxicity test methods, where properly followed, exhibit an acceptable range of variability. This variability is similar to test method variabilities exhibited by accepted analytical procedures for individual chemicals. (EPA 1985a) (DOE 1984).

Nevertheless, most of the available test methods need to have precision and accuracy determined through inter-laboratory calibration tests. Standardization of tests would increase their usefulness. Standard protocols and quality assurance procedures are essential for regulatory use.

Toxicity Persistence and Behaviour of Toxicants after Discharge.

As soon as an effluent mixes with water, its properties begin to change. The rate of change of toxicity is a measure of toxicity persistence. The level of toxicity may be reduced by dilution, or decreased by decay processes or compartmentalization processes or altered by the physical or chemical characteristics of the receiving environment. It has been suggested (DOE 1984) that better methods are needed to measure the compartmentalization and persistence of toxicity in the receiving water in order to accurately describe the environmental exposure occurring. On-site tests, however, indicate that toxicity is persistent in near-field zones and therefore toxicity degradation may not be important in permitting situations (EPA 1985a). Their position is to assume persistence unless reductions can be modelled/demonstrated, e.g. changes in toxicity after discharge can be assessed with bioassays using dilution water from upstream. Rapid chemical reactions and changes should be duplicated in the test. Ambient toxicity testing and artificial aging of an effluent can also be used to advantage.

Bioconcentration Test.
Tests exist to measure uptake into the animal body but better methods for measuring substances with high BCFs from unknown mixtures are needed both for wastewater and tissue (DOE 1984).

Toxicity Reduction Evaluation.
There are no standard methods to translate toxicity reductions into engineering controls on treating discharges to achieve regulatory requirements. Better methods are needed to enable one to locate the source of toxicity among the waste streams composing the final effluent. Particular attention must be given to comparisons involving treated final effluent and untreated component waste streams.

Converseley, it may be argued that treatment systems to remove toxicity can be designed as for other generic pollutants. Various treatment methods can be tried on various wastes to measure how good the removal is. Effluents can be fractionated using techniques that are directly relatable to the waste treatment methods that might be employed and then tested accordingly.

Effluent and Exposure Variability.
Variations in effluent flow and composition and variation in stream flow and quality are often large and unpredictable. The problems are not, however, greatly different from those associated with sampling for chemical analysis. Effluent variability can be handled by proper methods and frequency of sample collection. Exposure variability can be dealt with by either assuming a simple steady state exposure condition (with low flow conditions for receiving water and average effluent discharge rate) or alternatively, greater sophistication can be attained with statistical analysis and dynamic modelling.

Toxicant Interactions.
Multiple toxicants in a discharge can act together. Additivity appears to provide a reasonable estimate of the combined effects of individual acute toxic agents. Toxicities approaching the no-effect level do not appear additive, i.e. their potential for addition is reduced (EPA 1985a). Synergism is considered an extremely rare phenomenon (Roop 1985, EPA 1985a).

Acute to Chronic Ratio (ACR).
Chronic toxicity data should be used wherever possible. Where not available, an ACR value of 20 for non-persistent chemicals and 100 for persistent chemicals has been widely used, in both chemical-specific and whole effluent approaches to impact assessment. A value of 10 for whole effluents toxicity has been recently recommended by EPA.

Species Sensitivity Variability.
In setting regulatory requirements for effluents it is desirable to select test species which are sensitive to the toxicants, however, different species do exhibit a wide range of sensitivities. To best balance cost with toxicological certainty, EPA now recommends three species be tested to

eliminate a sufficient portion of the uncertainty in this factor (EPA 1985). Thus, a few well chosen test species can suffice, provided they represent different trophic levels and sensitivities. Guidance from EPA is that "Standard test species will be representative of the expected impact on resident species in most cases if a range of sensitivities is assessed and a sensitive test species identified. Resident species are assumed to be protected if the no-effect level for the sensitive test organism is not exceeded". Test species that resemble resident species with regard to feeding habits, physiology and behaviour are preferred (Roop 1985).

The Use of Ecological Field Surveys in Setting Permit Requirements.
Traditional field surveys of populations and communities of aquatic life do have a critical function: to provide insight into long term trends and the overall health of large reaches of water and the recovery of previously affected communities. Such surveys do, however, have a number of serious practical limitations as a regulatory tool:

- the cost to measure impact is likely 2-3 orders of magnitude higher than toxicity tests in all but the simplest situations
- in multiple discharge situations it is difficult to attribute effects to a single source
- only gross effects due to toxicity are detected so impacts must be large
- the impacts must have occurred already in order to measure them and therefore are not useful for treatment decisions on new discharges
- time required to complete surveys may be prohibitive
- the results are ambiguous insofar as translating them to treatment requirements.

Appendix 3

Appendix 3A. Biological Tests Conducted in EPS Aquatic Toxicology Laboratories, March, 1986

Appendix 3B. General Application of Biological Tests in EPS Aquatic Toxicity Laboratories as of March, 1986

Appendix 3C. Survey Inputs Classified According to "Application"

Appendix 3D. Use of Biological Tests and Procedures in Environmental Protection (DOE) Activities (to mid-1986)

Appendix 3E. Application of Biotests for Various Problem Sources

Appendix 3A. Biological Tests Conducted in EPS Aquatic Toxicology Laboratories, March 1986.

TEST	ORGANISM	RESPONSE/ASSESSMENT MEASURE	CLASS OF RESPONSE	REGIONAL CAPABILITY P	W	Q	A	N
Lethal								
Fish	rainbow trout Salmo	mortality (incapacitation[1])						
		short term/acute exposure	AL	0	0	0	0	0
		long term/chronic exposure	CL		X			
		hatch success/larval survival	CL	X			X	
	marine stickleback Gasterosteus	mortality (incapacitation[1])	AL	X			0	0
	other marine fish	mortality (incapacitation[1])	AL	X			X	X
Invertebrate	crustacean Daphnia	mortality (incapacitation[1])	AL	X	0		0	
	brine shrimp Artemia	mortality (incapacitation[1])	AL			X	X	
	clams, mussels	mortality	AL				X	X
	sand dollars	mortality	AL					X
Algae	Selenastrum (freshwater)	mortality	AL		X	X		
Sublethal								
Fish	rainbow trout Salmo	physiological stress; ATP level						
		short-term exposure (4 day)	AS			X		
		long-term exposure (21 day)	CS			X		
	other marine fish	physiological stress/MFO induction	AS				X	X
Invertebrate	crustacean Daphnia	reproduction/life cycle	CS		X		X	
	mussel Mytilus	behaviour/attachment	AS	X			X	
	clams, e.g. Macoma	behaviour/burrowing	AS				X	X
	sand dollars	behaviour/burrowing	S					X
Algae	Selenastrum (freshwater)	growth inhibition	AS		X	0	X	
		physiological stress (ATP level)	AS			X		
	Thalassiosira (marine)	growth inhibition	AS			X		
	Chlamydomonas	growth inhibition	AS			X		
Protozoan	Colpidium	growth inhibition	AS			X		
Bacteria	Photobacterium (Microtox)	metabolic; inhibition of light	AS	X	0	0		
	Salmonella (Ames test)	mutagenicity	AS/CS		0	X		
	Escherichia (chromotest)	initiation of genotoxic activity	AS/CS			0		
Bioaccumulation								
Fish	rainbow trout Salmo	bioaccumulation (short or long term exposure)	AS/CS			X	X	
Invertebrate	mussel Mytilus/clams Macoma	bioaccumulation (short or long term exposure)	AS/CS				X	X
Algae	Selenastrum	bioaccumulation (short term exposure)	AS			0		

[1] incapacitation is a sublethal effect, recorded in lethality tests

AL - acute lethal
CL - chronic lethal
AS - acute sublethal
CS - chronic sublethal

P - Pacific
W - Western and Northern
Q - Quebec
A - Atlantic
N - Newfoundland

0 - frequently used procedure

X - occasionally used procedure

Appendix 3B.

General Application of Biological Tests in EPS Aquatic Toxicity Laboratories as of March 1986.

	Fisheries Act	Ocean Dumping Control	Legal Tests	Effluent Monitoring	Dispersant Guidelines	OECD Testing	Pesticide Components	Offshore Activities	Spill Emergency Response	Tiered Ecotox Evaluation	In-Plant Studies	Wastewater Treatment Evaluation	Selected Investigations
Lethal Toxicity													
rainbow trout Salmo	X		X	X	X	X	X	X	X	X		X	X
stickleback Gasterosteus	X	X		X			X	X					X
other marine fish				X			X	X					X
crustacean Daphnia			X	X		X	X						X
brine shrimp Artemia		X							X	X			X
mussel, Mytilus		X					X						X
clams, Macoma and others		X					X						
algae Selenastrum	X			X		X				X			X
Sublethal Toxicity													
rainbow trout													
- physiological stress; ATP level	X			X					X	X		X	X
- reproduction, hatching success				X			X						X
other marine fish													
- physiological stress, MFO induction													X
crustacean Daphnia													
- reproduction, life cycle				X				X					X
mussel Mytilus													
- behaviour, attachment		X		X			X						X
clams Macoma													
- behaviour, burrowing		X						X					X
algae Selenastrum													
- growth inhibition	X			X		X				X	X	X	X
- physiological stress, ATP level	X			X		X			X				X
algae Thalassiosira													
- growth inhibition		X							X	X			X
algae Chlamydomonas													
- growth inhibition				X									X
protozoan Colpidium													
- growth inhibition									X	X	X	X	X
bacteria Photobacteria													
- Microtox test (metabolic inhibition)	X			X		X			X	X	X	X	X
bacteria Salmonella													
- Ames test for mutagenicity		X		X						X			X
bacteria Escherichia													
- chromotest for genotoxicity		X		X		X			X	X	X	X	X
Bioaccumulation													
rainbow trout Salmo							X						X
mussel Mytilus		X					X						X
clam Macoma		X						X					X
algae	X			X		X			X	X		X	X

Appendix 3C. Survey Inputs Classified According to 'Application'

Application Number	I.D. Number of Addendum A (Use Survey)
1.	T21, T25, E6, I3, I23, (Q1), (Q2), Q5, A7, A11, A12, A18, A19, A20, A29, W17, P16, P30, P31
2.	T26
3.	T22, T23, (T25), C1, C2, I10, I11, I12, I17, I19, (I23), O3, O5, Q1, Q2, Q4, Q6, A3, A4, A25, W6, W8, W13, P1, (P2), P5, (P7), (P8a), P15, P15a, P17, P18, P22, (P30)
4.	T24, P2, P4, (P7)
5.	(A1), A9, A24
6.	T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, I5, I7, I8, I9, I18, I20, I21, Q7, A14, A16, W7, W12, W14, P13
7.	E4, (I14), (I18), O3, A1, A5, A9, (A24), W2, P2, P7, P11, P14, Q3
8.	T27, T28, E1, E5, (C1), I1, I6, I14, (I19), I24, O1, O6, A21, Q3
9.	O7, (Q8), Q9, A6, W3, P20, P29
10.	E2, I2, I4, I15, O2, Q3, Q8, A2, A15, A17, W4, P21
11.	(T24), T29, E3, I5, I22, O4, O8, Q10, A18, A10, A13, A26, A28, W1, W5, W11, W13, P3, P6, P8, P9, P10, (P13), P19, P23, P28
12.	O9, W10

QA/QC or Methods Development

I16, O1, A22, A23, A27, W9, W15, W16, P11, P12, P14, P24, P25, P26, P27, P32, P33, P34, P35, P36

NOTE: The above application number (1-12) corresponds to the general applications for biological testing defined in Chapter 3.3. The i.d. numbers on the right are references to questionnaire responses contained in Addendum I. Those responses were 'fitted' to an application to show the distribution of the various uses identified.

Appendix 3D Use of Biological Tests and Procedures in Environmental Protection (DOE) Activities (to mid-1986).

TOXICITY TESTING				Identify/Screen Potential Hazards	Signal Changes in Env. Quality	Establish Priorities	Assess Hazards	Assess Environmental Effects	Set Ambient Env. Quality Objective	Evaluate Waste Treatment Methods	Apply Site-Specific Controls	Apply Assessment-Control Schemes	Apply National Level Protection	Enforce Regulatory Standards	Monitor Compliance to Controls	Monitor Effectiveness of Controls	Monitor Long Term Trends
Organism/Test		Assessment Measure															
Lethal Fish	rainbow trout Salmo	mortality[1]	A/C	X		X	X		X	X	X	X	X	X	X	X	
		hatch success	C				X		X							X	
	marine stickleback	mortality[1]	A	X		X	X		X		X		X	X		X	
	salmon fry	mortality[1]	A			X	X		X		X		X	X		X	
	indigenous fish[2]	mortality[1]	A			X	X		X		X		X	X		X	
Invertebrates	Daphnia	mortality[1]	A	X		X	X		X	X	X	X			X	X	
	brine shrimp Artemia	mortality[1]	A	X		X	X		X					X		X	
	clams, mussels	mortality[1]	A			X	X		X		X					X	
	amphipods	mortality[1]	A				X										
	other indigenous invert	mortality	A/C				X										
Algae	Selenastrum	mortality	A	X		X	X		X		X					X	
Sublethal Fish	rainbow trout Salmo	ATP-stress	A/C			X	X			X				X		X	
	indigenous fish[7]																
Invertebrates	Daphnia	reproduction	C			X	X		X		X	X				X	
	indigenous invert[5]	assorted[6]	A/C				X										
Algae	Selenastrum	growth inhibition	A	X		X	X		X	X	X	X		X		X	
		ATP-stress	A			X	X									X	
	Thalassiosira	growth inhibition	A			X	X										
	Chlamydomonas	growth inhibition	A				X										
Protozoa	Colpidium	growth inhibition	A			X	X			X							
Bacteria	Microtox	metabolic/light inhib	A	X		X	X			X	X	X		X	X	X	
	chromotest	genotoxic activity	A/C	X		X	X			X	X	X				X	
	Ames test	mutagenicity	A/C			X	X				X					X	
Bioaccumulation Fish	rainbow trout	body burden	A/C			X	X				X					X	
Invertebrates	clams/mussels	body burden	A/C			X	X				X					X	
	other indigenous species	body burden	A/C				X										
Algae	Selenastrum	body burden	A			X	X				X	X				X	
Biodegradability	mixed bacteria culture					X	X										

FIELD BIOMONITORING				Identify/Screen Potential Hazards	Signal Changes in Env. Quality	Establish Priorities	Assess Hazards	Assess Environmental Effects	Set Ambient Env. Quality Objective	Evaluate Waste Treatment Methods	Apply Site-Specific Controls	Apply Assessment-Control Schemes	Apply National Level Protection	Enforce Regulatory Standards	Monitor Compliance to Controls	Monitor Effectiveness of Controls	Monitor Long Term Trends
Approach	Organism	Assessment Measure															
insitu assays	indigenous fish[4]/invert[5]	lethality		X	X	X		X			X					X	
		sublethal effects[3]			X	X		X			X					X	
		contaminant uptake			X	X		X			X					X	
	benthic invert community	colonization						X			X					X	
contaminant residues	endemic fish/invert	body burdens			X	X		X			X					X	X
functional biomon.	endemic fish/invert	sublethal effects[3]						X			X					X	X
structural biomon.	endemic populations	abundance/diversity						X			X					X	X

A = acute exposure C = chronic exposure

1. includes incapacitation
2. e.g. herring larvae
3. may include MFO induction, metalothionein assays, anaphase aberation tests, tissue disorders, histopathological analysis, other physiological responses
4. such as salmon fry, herring larvae, stream invertebrates and fish
5. such as amphipods, clams, other benthic fauna
6. including respiration, behaviour, scope for growth, recruitment
7. e.g. cod-egg assay.

Appendix 3E.

Applications of Biotests for Various Problem Sources, According to Use-Survey (Addendum I)

	Liquid Effluents	Residues/Sludges/Leachates	Hazardous Wastes	Spill-Related Products	Pesticides	Chemicals	Ocean Dumping	Aquatic Sediments	Emissions	Offshore Oil & Gas	Multiple Source	New Developments
Identify/Screen Potential Hazards	X	X	X	X		X	X		X			
Signal Changes in Env. Quality												
Establish Priorities	X		X	X		X						
Assess Hazards	X	X	X	X	X	X		X		X	X	
Assess Environmental Effects	X			X		X					X	
Set Ambient Env. Quality Objective										X		X
Evaluate Waste Treatment Methods	X	X										
Apply Site-Specific Controls	X				X		X	X		X	X	X
Apply Assessment-Control Schemes			X					X				
Apply National Level Protection	X		X	X		X	X					
Enforce Regulatory Standards	X			X								
Monitor Compliance to Controls	X					X	X	X		X		
Monitor Effectiveness of Controls	X		X	X	X		X	X				X
Monitor Long Term Trends						X						

Appendix 4

Appendix 4A.	Biological Tests[1] being Evaluated[2] for Use in Association with the Ocean Dumping Control Act
Appendix 4B.	MPD Ecological Data Components
Appendix 4C.	Tests Recommended in "OECD Guidelines for Testing of Chemicals" (1981)
Appendix 4D.	Freshwater and Marine Species Recommended by U.S. EPA for Acute Lethal Toxicity Determination of Effluents (from EPA 1985b)
Appendix 4E.	Primary Chronic Aquatic Toxicity Tests Recommended by U.S. EPA
Appendix 4F.	Use of Genotoxic Assays in Region V - U.S. EPA
Appendix 4G	Biological Tests being Evaluated in the Ontario MISA Program
Appendix 4H.	EP Pacific Region: Aquatic Toxicity Tests[1] Used/ Desirable[2] for Specialized Regional Needs (1/12/86)
Appendix 4I.	EP Atlantic Region: Aquatic Toxicity Tests[1] Used/ Desirable[2] for Specialized Regional Needs (1/12/86)
Appendix 4J.	EP Newfoundland District: Aquatic Toxicity Tests[1] Used/ Desirable[2] for Specialized Regional Needs (1/12/86)
Appendix 4K.	EP Quebec Region: Aquatic Toxicity Tests[1] Used/ Desirable[2] for Specialized Regional Needs (1/12/86)
Appendix 4L.	EP Ontario Region: Aquatic Toxicity Tests[1] Used/ Desirable[2] for Specialized Regional Needs (1/12/86)
Appendix 4M.	Potential Applications of Field Biomonitoring

Appendix 4A. Biological Tests[1] being Evaluated[2] for Use in Association with the Ocean Dumping Control Act.

Amphipod sediment assay (10 day) with *Rhepoxinius abronius*

Benthic community recruitment bioassay

Sediment phase tests (for bioaccumulation) with the clam *Macoma balthica*

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1. as recommended in Envirochem (1986). "Feasibility Assessment of Sediment Toxicity Tests Suitable for ODCA Application Review". Prepared for the P&Y Regional Ocean Dumping Advisory Committee, March 1986.

2. as supported for 1987-88 by ODCA program managers.

Appendix 4B. MPD Ecological Data Components

Ecological Data Components (modified from OECD-MPD)
Suggested for Notification Requirements by
Environmental Contaminants Act Amendments
Consultative Committee[1]

- a fish LC_{50}
- Daphnia LC_{50}
- partition coefficient, water solubility, hydrolysis
- biodegradation, one of OECD test 301 A-E
- bioaccumulation, one of OECD test 305 A-E

Ecotoxicity Data Components for the OECD
Minimum Pre-Marketing Set of Data (taken from [1] which references [2] as source)

- Fish LC_{50} - at least 96 hours exposure
- Daphnia - reproduction 14 days
- Algae - growth inhibition 4 days

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1. Final Report of Environmental Contaminants Act Amendments Consultative Committee. October 1986. Ottawa. DSS Cat No. En.40-343/1986-E.

2. Organization for Economic Co-operation and Development, Decision of the Council (8th December, 1982) Concerning the Minimum Pre-Marketing Set of data in the Assessment of Chemicals, C(32) 196 (Final)

Appendix 4C. Tests Recommended in "OECD Guidelines for Testing of Chemicals" (1981)

Section 2. Effects on Biotic Systems (i.e. Ecotoxicity) Updated guidelines adopted 1984.

- acute lethal fish test *
- chronic 14-day fish test*
- algal growth inhibition test*
- Daphnia immobilization test*
- Daphnia reproduction test*
- avian dietary test
- avian reproduction test
- earthworm acute toxicity test
- terrestrial plant growth test
- activated sludge respiration inhibition test

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* included in the set of national core aquatic toxicity tests for Canada (Sergy 1987)

Appendix 4D. Freshwater and Marine Species Recommended by U.S. EPA for Acute Lethal Toxicity Determination of Effluents (from EPA 1985b)

Freshwater	
Vertebrates	
Cold Water	
Brook trout:	Salvelinus fontinalis
Coho Salmon:	Oncorhynchus kisutch
Rainbow trout:	Salmo gairdneri
Warm Water	
Bluegill:	Lepomis macrochirus
Channel catfish:	Ictalurus punctatus
Fathead minnow:	Pimephales promelas
Invertebrates	
Cold Water	
Stoneflies:	Pteronarcys spp.
Crayfish:	Pacifastacus leniusculus
Mayflies:	Baetis spp. or Ephemerella spp.
Warm Water	
Amphipods:	Hyella, spp., Gammarus lacustris, G. Fasciatus, or G. pseudolimnaeus
Cladocera:	Daphnia magna or D. pulex, Ceriodaphnia spp.
Crayfish:	Orconecates spp., Cambarus spp., Procambarus spp.
Mayflies:	Hexagenia limbata or H. bilineata
Midges:	Chironomus spp.

Marine and Estuarine	
Vertebrates	
Cold Water	
English sole:	Parophrys vetulus
Sanddab:	Citharichthys stigmaeus
Winter flounder:	Pseudopleuronectes americanus
Warm Water	
Flounder:	Paralichthys dentatus, P. Lethostigma
Longnose Killifish:	Fundulus similis
Mummichog:	Fundulus heteroclitus
Pinfish:	Lagodon rhomboides
Sheepshead minnow:	Cyprinodon variegatus
Silverside:	Menidia spp.
Spot:	Leiostomus xanthurus
Threespine stickleback:	Gasterosteus aculeatus
Invertebrates	
Cold Water	
Dungeness crab:	Cancer magister
Oceanic shrimp:	Pandalus jordani
Green sea urchin:	Strongylocentrotus droebachiensis
Purple " " :	S. purpuratus
Sand dollar:	Dendraster excentricus
Warm Water	
Blue crab:	Callinectes sapidus
Mysid:	Mysidopsis spp., Neomysis spp,
Grass shrimp:	Palaemonetes spp.
Penaid shrimp:	Penaeus setiferus, P. duorarum, P. aztecus
Sand shrimp:	Crangon spp.
Pacific oyster:	Crassostrea gigas
American oyster:	Crassostrea virginica

Appendix 4E. Primary Chronic Aquatic Toxicity Tests Recommended by U.S. EPA.

(see EPA 1985c)

1.	Fathead Minnow; *Pimephales promelas*	- Growth and Survival
2.	" " " "	- Larval Teratogenicity
3.	Waterflea; *Ceriodaphnia* sp.	- Reproduction (and Immobilization)
4.	Green Algae; *Selenastrum* sp.	- Growth Inhibition

Appendix 4F. Use of Genotoxic Assays in Region V - U.S. EPA*.

Genotoxicity Assays:

- Ames Test
- SOS Signal Induction
- Sister Chromatid Exchange
- Aneuploidy
- DNA Adducts
- Enzyme Induction
- Urine Mutagenicity
- Pathology

Currently, the Ames test is being used in Region V to screen effluents, and not as a permit monitoring requirement. As a result of these screens, more detailed chemical analyses are, however, being required by permit.

* from presentation on U.S. EPA Region V Approach for the Control of Wastewater Toxics Using the NPDES Permit System. November 26, 1986. J.D. Giattina and L. Anderson-Carnahan.

Appendix 4G. Biological Tests being Evaluated in the Ontario MISA Program

(a) Lethal-Sublethal

1. acute lethality in rainbow trout*
2. caged fish (fathead minnows or trout)
3. microcosm system with fathead minnows, Daphnia, clams, amphipods, mayflies
4. benthic community analysis
5. growth inhibition in Chlorella fusca
6. Daphnia immobilization*
7. rainbow trout respiration
8. growth inhibition in fathead minnow larvae
9. rainbow trout avoidance

(b) Genotoxicity

a battery of genotoxicity tests

(c) Contaminant Residues

1. natural fish and benthos body burdens
2. caged fish, clams body burdens

(d) Additions by DOE

1. Microtox
2. Algal toxicity microtest with Selenastrum
3. Genotoxic accumulation and/or transformation

* expected to see major use

Appendix 4H. EP Pacific Region: Aquatic Toxicity Tests[1] Used/Desirable[2] for Specialized Regional Needs (1/12/86)

Test Type	Organism	Response	Test Status	Remarks
Lethality	Pacific salmon (Coho, Chinook, Sockeye, Chum, Pink) - various life stages	mortality	occasional use	indigenous marine commercial fish
	Herring larvae	mortality	occasional/seasonal	indigenous marine commercial fish
	Cod eggs	hatching success	desirable/seasonal	" " " "
	Whitefish	mortality	desirable	indigenous freshwater fish
Other	Anaphase Aberration Sediment Assay			to characterize genotoxicity of marine sediments
	Freshwater Sediment Assay			
	Macoma Marine Sediment Assay	death, behaviour, bioaccumulation		

[1] not included in national core set
[2] identified by respective regional staff

Appendix 4I. EP Atlantic Region: Aquatic Toxicity Tests[1] Used/Desirable[2] for Specialized Regional Needs (1/12/86)

Test Type	Organism	Response	Test Status	Remarks
Lethality	Salvelinus fontinalis (brook trout fingerlings)	mortality	occasional use	used as freshwater indigenous species for legal tests and site-specific water quality criteria
	Salmo gairdneri (rainbow trout eggs and larvae)	hatching success and larval survival	occasional use	sensitive life stage
	Atlantic salmon	lethality	-	desirable as indigenous fish species
	Pseudopleuronectes americanus (flounder)	mortality	occasional use	marine indigenous commercial benthic fish useful for sediment work
	asst. clams and mussels	mortality	occasional use	indigenous invertebrate; benthic epi or infauna
Sublethality	P. americanus (flounder)	MFO induction	occasional use	(see above)
	asst. clams and mussels	burrowing and byssal attachment	occasional use	(see above)
Bioavailability	S. gairdneri (rainbow trout)	contaminant accumulation	occasional use	
	Mytilus edulis (mussel)	contaminant accumulation	occasional use	marine indigenous commercial invertebrate

[1] not included in national core set
[2] identified by respective regional staff

Appendix 4J. EP Newfoundland District: Aquatic Toxicity Tests[1] Used/Desirable[2] for Specialized Regional Needs (1/12/86)

Test Type	Organism	Response	Test Status	Remarks
Lethality	Macoma sp. (clam)	mortality	in use	marine indigenous benthic invertebrate
	Echinarachnius sp. (sand dollar)	mortality	in use	marine indigenous benthic intertebrate useful for sediment work
Sublethality	Gasterosteus aculeatus (3 spine stickleback)	MFO induction	in use	marine indigenous fish useful for sediment work
	Macoma sp.	burrowing behaviour	in use	marine indigenous benthic invertebrate useful for sediment work
	Echinarachnius sp. (sand dollar)	burrowing behaviour	in use	marine indigenous benthic invertebrate useful for sediment work

[1] not included in national core set
[2] identified by respective regional staff

Appendix 4K. EP Quebec Region: Aquatic Toxicity Tests[1] Used/Desirable[2] for Specialized Regional Needs (1/12/86)

Test Type	Organism	Response	Test Status	Remarks
Lethality	*S. capricornutum* (green algae)	mortality	under development	Routine use of an algal post-exposure viability test with one year
Sublethality	*S. capricornutum* (green algae)	4 hour ATP stress	Routine use is established	—
	A. nauplii (brine shrimp)	ATP stress	under development	Capability for routine use expected within one year
	Daphnia sp.	reproduction/ immobilization	when available from Belgium, a maintenance-free cyst culture	
	Photobacterium sp. ("Super bug")	luminescence inhibition	under development by Microbics Corporation	Commercialization expected in 1987: capacity for routine use expected afterwards
Genotoxicity	Bioengineered bacteria *E. coli* ("dark mutant")	mutagenetic potential	(as above)	(as above)
	S. capricornutum (green algae)	contaminant accumulation	Inorganic bioaccumulation protoc. is establ.	Capability for routine application is available now
			Organic bioacc. protocol is being investigated	Capability for routine use expected within one year
			Genotoxic bioacc. potential is being investig.	Capacility for routine use expected thin 6 months
Biodegradability	Mixed seed bacteria		Routine use is established	—

[1] not included in national core set
[2] identified by respective regional staff

Appendix 4L. EP Ontario Region: Aquatic Toxicity Tests[1] Used/Desirable[2] for Specialized Regional Needs (1/12/86)

Test Type	Organism	Response	Test Status	Remarks
	Fathead Minnow (Pimephales)	Lethality		) Test battery being recommended
	Amphipod (Hyalella)	Lethality		) for Great Lakes sediment toxicity
	Mayfly (Hexagenia)	Lethality		) characterization.
	Waterflea (Daphnia)	Lethality		)
	Earthworm (Lumbricus)	Lethality		)

[1] not included in national core set
[2] identified by respective regional staff

Appendix 4M.

Potential Applications of Field Biomonitoring

Approach	Organism	Assessment Measure	Identify/Screen Potential Hazards	Signal Changes in Env. Quality	Establish Priorities	Assess Environmental Effects	Apply Site-Specific Controls	Monitor Effectiveness of Controls	Monitor Long Term Trends
insitu assays	indigenous fish[4]/invert[5]	lethality	X	X	X	X	X	X	
		sublethal effects[3]		X	X	X	X	X	
		contaminant uptake		X	X	X	X	X	
	benthic invert community	colonization				X	X	X	
contaminant residues	endemic fish/invert	body burdens		X	X	X	X	X	X
functional biomon.	endemic fish/invert	sublethal effects[3]				X	X	X	X
structural biomon.	endemic populations	abundance/diversity				X	X	X	X

3. may include MFO induction, metallothionein assays, anaphase aberration tests, tissue disorders, histopathological analysis, other physiological responses
4. such as salmon fry, herring larvae, stream invertebrates and fish
5. such as amphipods, clams, other benthic fauna

Appendix 6

Appendix 6A. Estimated Actual Cost for Toxicity Tests: EP Laboratories Dartmouth

Appendix 6B. Estimated Actual Cost for Toxicity Tests: EP Laboratories Vancouver

Appendix 6C. Estimated Cost and Operational Variables for Toxicity Tests: EP Laboratories Montreal

Appendix 6D. Costs of Toxicity Tests in the Canadian Private Sector (Jan 87)

Appendix 6E. Cost Summary of Laboratory Toxicity Tests in EPA Region IV (in $ U.S.)

Appendix 6A. Estimated Actual Cost for Toxicity Tests
EP Laboratories, Dartmouth

PROCEDURE	COST IN $
Rainbow Trout Acute Lethality	
96 hr. LC_{50} static	305.00
96 hr. compliance flow-thru	560.00
Stickleback (as per trout)	
Artemia Acute Lethality 24 hr.	180.00
Daphnia Acute Lethality 48 hr.	280.00
Daphnia Reproduction 21 day life cycle	1600.00
Macoma Bioaccumulation 30 day	770.00

Includes O&M, salary for tests and maintenance, preliminary analysis, capital (10 year depreciation).

Does not include space rental, utilities, reporting or chemical analysis.

Appendix 6B. Estimated Actual Cost for Toxicity Tests, EP Laboratories, Vancouver

PROCEDURE	COST IN $	
	Non-Legal	Legal*
Rainbow Trout Acute Lethality		
96 hr. LT_{50} static	290.00	710.00
96 hr. LC_{50} static	450.00	1200.00
96 hr. LC_{50} flowthru	2200.00	2500.00
Daphnia Acute Lethality	170.00	N/A
Microtox EC_{50}	100.00	N/A

Reflects total cost of operation and as used for external agency billing rate.

Includes O&M, salary for tests and maintenance, capital as well as rent, utilities and reporting.

* samples analyzed with intent of being used as evidence in court

Appendix 6C. Estimated Cost and Operational Variables for Toxicity Tests
EP Laboratories, Montreal (from Bermingham 1987)

PROCEDURE AND REQUIREMENTS:			COST PER SAMPLE		
			element	1 sample/batch	5 sample/batch
	Space:	150m^2	Space	90	36
Lethality	Capital:	40K	Capital	27	11
Trout - Salmo	TTLR:	M	O & M	40	40
96 hour LC$_{50}$	p/space:	5	Salary	240	240
8 dilutions @ 10 fish	ODB:	2			
	pd/test:	2	Total	$397	$327
	Space:	150m^2	Space	90	18
Lethality	Capital:	40K	Capital	27	5
Trout - Salmo	TTLR:	M	O & M	12	6
96 hour LT$_{50}$ or	p/space:	5	Salary	48	42
explor. LC$_{50}$ (3 dil.@ 3 fish)	ODB:	12			
	pd/test:	0.4	Total	$177	$ 71
	Space:	as	Space		
Lethality	Capital:		Capital	as per	as per
Stickleback - Gasterosteus	TTLR:	per	O & M	trout	trout
96 hour LC$_{50}$; LT$_{50}$	p/space:		Salary		
	ODB:	trout			
**	pd/test:		Total		
	Space:	25m^2	Space	15	5
Lethality/Immobility	Capital:	5K	Capital	3	1
Water Flea - Daphnia	TTLR:	MH	O & M	6	2
48 hour	p/space:	3	Salary	140	140
	ODB: .	3			
**	pd/test:	1	Total	$164	$148
	Space:	15m^2	Space	9	5
Lethality	Capital:	5K	Capital	3	2
Brine Shrimp - Artemia	TTLR:	ML	O & M	4	2
24 hour	p/space:	2	Salary	100	100
	ODB:	2			
	pd/test:	1	Total	$116	$109
	Space:	25m^2	Space	23	23
Lethality	Capital:	15K	Capital	16	16
Amphipod - Rhepoxinius	TTLR:	MH	O & M	6	6
10 day sediment assay	p/space:	2	Salary	420	420
	ODB:	0.66			
**	pd/test:	3	Total	$465	$465

PROCEDURE AND REQUIREMENTS:			COST PER SAMPLE		
			element	1 sample/batch	5 sample/batch
	Space:	$150m^2$	Space	90	72
ATP Energy Stress & Lethality	Capital:	55K	Capital	38	30
Trout - Salmo	TTLR:	L&H	O & M	180	180
96 hour	p/space:	5	Salary	480	480
12 dilutions @ 10 fish	ODB:	1			
	pd/test:	4	Total	$788	$762
	Space:	15	Space	103	103
Larval Growth Inhibition	Capital:	10	Capital	69	69
Fathead Minnow - Pimephales	TTLR:	L&H	O & M	30	30
7 day	p/space:	2	Salary	2400	2400
	ODB:	0.1			
**	pd/test:	20	Total	2602	2602
	Space:	$30m^2$	Space	56	56
Reproduction & Immobility	Capital:	5K	Capital	11	11
Daphnia	TTLR:	L&H	O & M	6	6
21 day	p/space:	3	Salary	1080	1080
	ODB:	0.3			
**	pd/test:	9	Total	$1153	$1153
	Space:	$15m^2$	Space	9	2
Growth Inhibition	Capital:	15K	Capital	10	2
Algae - Selenastrum	TTLR:	ML	O & M	3	2
4 day microplate technique	p/space:	2	Salary	25	10
	ODB:	20			
	pd/test:	0.1	Total	$ 47	$ 16
	Space:	$35m^2$	Space	21	7
Growth Inhibition	Capital:	15K	Capital	10	3
Algae - Selenastrum	TTLR:	M	O & M	6	2
8 day conventional technique	p/space:	3	Salary	120	120
	ODB:	3			
	pd/test:	1	Total	$157	$132
	Space:	$5m^2$	Space	3	1
Light Emission Inhibition	Capital:	15K	Capital	10	2
Microtox test	TTLR:	M	O & M	50	10
5-15 min.	p/space:	1	Salary	30	20
	ODB:	7			
	pd/test:	0.15	Total	$ 93	$ 33

PROCEDURE AND REQUIREMENTS:			COST PER SAMPLE		
			element	1 sample/batch	5 sample/batch
	Space:	$20m^2$	Space	12	3
Genotoxic Potential	Capital:	15K	Capital	10	2
SOS Chromotest with S9	TTLR:	MH	O & M	400	80
2 hour	p/space:	2	Salary	40	28
	ODB:	10			
	pd/test:	0.2	Total	$462	$113
	Space:	$30m^2$	Space	18	5
Mutagenic Reversion	Capital:	15K	Capital	10	2
AMES Test	TTLR:	H	O & M	75	75
4 day fluctuation test	p/space:	2	Salary	80	80
	ODB:	4			
	pd/test:	0.5	Total	$183	$162
	Space:	$150m^2$	Space	450	230
Bioaccumulation	Capital:	40K	Capital	140	70
Clam - Macoma	TTLR:	H&L	O & M	100	100
30 day	p/space:	5	Salary	720	720
	OWB:	2			
**	pd/test:	6	Total	$1410	$1120

key:

TTLR = Technician Training Level Required
ODB/OWB = Optimum Daily/Weekly Batch
p = person
pd = person/day

** this test is not in Montreal lab but estimated from data of other labs

Bermingham, N. 1987. Personal Communication of Unpublished Work. Environment Canada, Montreal.

Explanatory Notes to Appendix 6C.

1. Five (5) technician training levels (TTLR) were defined and matched with appropriate annual salaries. Those were:
   H = $160 per day - 40,000 per year
   MH = $140 " " - 35,000 " "
   M = $120 " " - 30,000 " "
   ML = $100 " " - 25,000 " "
   L = $ 80 " " - 20,000 " "

2. Space in (m^2) includes total requirements for all phases of that test. Assumptions of operational space were made for various types of tests and that figure used for all tests in that type, e.g. 150 m^2 was estimated to house a fish lab (wet lab).

3. Capital equipment costs were estimated from average values on the LMC (Lab Managers' Committee) lists and includes all equipment necessary to operate the facility type identified in (2) above plus any additional special equipment for that particular test, e.g. 40K was estimated for basic operation of the fish lab (wet lab).

4. In cost per test calculations:

   - space rental assumes a rate of $150.00 per meter per year

   - capital is depreciated over a 10 year period, 52 payments per year, 12% annual interest

   - O&M includes all expendable supplies

   - effort = $ based on TTLR salary and pd/test.

5. Quality control is assumed at 10-15%.

6. Cost figures do not reflect savings realized in multi-procedure capability via shared space and equipment.

Appendix 6D. Costs of Toxicity Tests in the Canadian Private Sector (Jan 87)

PROCEDURE	1 test batch * Average (range)	10 test batch * Average (range)
Fish Acute Lethality		
96 hour LC_{50} static	383 (205-450)	272 (190-370)
96 hour continuous flow	773 (680-865)	665 (540-790)
96 hour LT_{50} static	208 (110-400)	150
Daphnia Acute Lethality		
24 hour static (Daphnia or Artemia)	193 (180-200)	164 (150-180)
48 hour static	250	200
48 hour continuous flow	580	460
96 hour LC_{50}	600	450
Amphipod Sediment Assay (10 day)	760	446
Fathead Minnow Larval Growth (7 day)	1000	800
Daphnia 21 day life cycle (ASTM)	975 (750-1200)	780 (600-960)
Ceriodaphnia 7 day life cycle (EPA)	715 (650-780)	586 (550-625)
Algae Growth Inhibition		
4 day microplate technique	180	125
8 day conventional technique	718 (695-750)	600 (555-645)
21 day technique	840	680
Microtox	113 (100-125)	100
Ames Test	1200 (1000-1400)	
Chromotest	300	250

* n ranges from 1 to 6

Appendix 6E. Cost Summary of Laboratory Toxicity Tests in EPA Region IV* (in $ U.S.)

TEST CATEGORY	AVERAGE AND RANGE	n
Acute **		
24 hr. static	237 (50- 500)	26
48 hr. static	462 (150- 950)	24
48-96 hr. flowthru	1361 (400-4000)	14
Chronic		
Ceriodaphnia 7 day life cycle	1173 (300-2200)	11
Fathead Minnow 7 day larval growth	1400 (500-1850)	8
Fathead Minnow 8 day (egg and larval stage)	1642 (800-2000)	6
Algal Growth 96 hr.	688 (200-1400)	8

* personal communication and data from Bill Peltier, United States environmental Protection Agency Region IV.

** Survey requested cost for most common static tests used which are fathead minnow and Daphnia acute lethality.

Appendix 7

Appendix 7A List* of Protocols and Procecures for each Core Aquatic Toxicity Tests

Part A Acute Lethality Testing Procedures for *Salmo gairdneri*

Part B Acute Lethality Testing Procedures for *Gasterosteus aculeatus*

Part C Acute Lethality/Immobility Testing Procedures for *Daphnia magna*

Part D Acute Lethality Testing Procedure for *Artemia sp.*

Part E Acute Lethality Testing Procedures for Marine Amphipods

Part F Sublethal ATP Energy Stress Testing Procedure for *Salmo gairdneri*

Part G Sublethal Testing Procedures for *Pimepales promelas*

Part H Sublethal Testing Procedures for *Daphnia sp.*/*Ceriodaphnia sp.*

Part I Sublethal Growth Inhibition/Stimulation Testing Procedures for Unicellular Green Alga

Part J Sublethal Testing Procedures for Bacterial Luminescence Inhibition

Part K Genotoxicity Testing Procedures for Bacteria

Part L Bioconcentration Testing Procedures for Fish

Part M Bioavailability Test Procedures for Marine Molluscs

Part N Biodegradation Testing Procedures

* the above constitutes a table of contents for Addendum 2 which contains copies of each protocol listed

Appendix 7A; Part A

ACUTE LETHALITY TESTING PROCEDURES FOR SALMO GAIRDNERI

Procedure	Test Type	End Point	Legal Toxicity Requirements	Frequency of Testing
° Canadian Meat and Poultry Products Plant Liquid Effluent R & G's	C.F. & S	96 hr P/F 50% surv. in 100%	only new and expanded plants	4 / yr or 1 / yr
° Canadian Potato Processing Plant Liquid Effluent R & G's	C.F. & S	96 hr. P/F 50% surv. in 100%	guideline only	no freq.
° Canadian Pulp and Paper Effluent R & G's	C.F. & S	96 hr. P/F 80% surv. in 65%/35%	only new, altered & expanded mills	no freq.
° Canadian Metal Mining Liquid Effluent R & G's	C.F. & S	96 hr. P/F 50% surv.	guideline only	no freq.
° Canadian Petroleum Refinery Effluent R & G's	C.F. & S	96 hr & 24 hr., 50% surv. in 100%,	all plants audit occasionally	infreq. (ie 1/yr)
		24 hr. static, 50% surv. in 100%	all plants report monitoring	1/month
° Canadian Guidelines on the Use and Acceptability of Oil Spill Dispersants	S	96 hr. LC50 (3 end-points)	guideline	as required
° Canadian Standard Procedure for Testing the Acute Lethality of Liquid Effluents	S, SR, OR CF	96 hr. P/F 50% surv. in 100%	guideline	-
° APHA - Standard Methods for the Examination of Water and Wastewaters - 810: Toxicity Test Procedures for Fish, 16th Edition, 1985	S, SR, OR CF	96 hr. LC50 96 hr. EC50	procedure outline	-
° U.S. Food and Drug Administration, Freshwater Fish Acute Toxicity Technical Guide	S	96 hr. LC50	procedure outline	-
° Sprague's ABC's of Pollutant Bioassay Using Fish ASTM/STP528	S, SR, OR CF	96 hr. LC50 ET 50	procedure outline	-

ACUTE LETHALITY TESTING PROCEDURES FOR SALMO GAIRDNERI (Continued)

Procedure	Test Type	End Point	Legal Toxicity Requirements	Frequency of Testing
° U.S. EPA Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (3rdEdition)	S, SR, OR CF	96 hr. LC50 Acute NOEC	procedure outline	-
° OECD Guidelines for Testing of Chemicals - Fish Acute Toxicity Test	S, SR, OR CF	96 hr. LC50	procedure outline	-
° British Health and Safety Commission - Approved Test Method for Acute Lethal Toxicity to Fish	S, SR, OR CF	96 hr. LC50	procedure outline	-
° British Dept. of Environment Standard Operating Procedure for Acute Toxicity to Fish	CF	96 hr. LC50	procedure outline	-
° Province of British Columbia Guidelines and Laboratory Procedures for Measuing Acute Lethal Toxicity of Liquid Effluents to Fish	S, SR, OR CF	96 hr. P/F 96 hr. LC50 LT 50 Toxic Units	guideline	-
° Province of Alberta Procedures Manual for Acute Lethality Fish Bioassays	S	96 hr. LC50	guideline	-
° Province of Ontario Protocol to Determine the Acute Lethality of Liquid Effluents to Fish	S, SR, OR CF	96 hr. LC50	guideline	-

P/F = pass/fail, S = Static, SR = Static replacement, CF = continuous flow (LC50, EC50 and TLm are equivalent)

Appendix 7A; Part B.

ACUTE LETHALITY TESTING PROCEDURE FOR GASTEROSTEUS ACULEATUS

Procedure	Test Type	End-Point
° Environment Canada, Atlantic Region - Laboratory Procedure for Determining the Acute Lethality of Oil-Based Drilling Fluids to Threespine Stickleback	S	96 hr. LC50
° Fisheries and Oceans Canada - Threespine Stickleback Acute Lethality Procedure for Waste Drilling Fluids	S	96 hr. LC50

S = Static

Appendix 7A; Part C.

ACUTE LETHALITY/IMMOBILITY TESTING PROCEDURES FOR DAPHNIA MAGNA

Procedure	Test Type	Recommended End-Point
° APHA - Standard Methods for the Examination of Water and Wastewater - 804: Toxicity Test Procedures for *Daphnia*, 16th Edition, 1985	S	lethality (96 hr. LC50) immobility (96 hr. EC50)
° ISO - International Orgtanization for Standardization - Method for Determination of the Inhibition of the Mobility of *Daphnia Magna* Straus	S	immobility (24 hr. EC50) (48 hr. EC50)
° ASTM - Standard Static Acute Toxicity Tests on Wastewaters with *Daphnia*, Annual Book of ASTM Standards, 1986	S	lethality (48 hr. LC50) immobility (48 hr. EC50)
° OECD Guidelines for Testing of Chemicals: Range-Finding 24 hr. EC50 Text	S	immobility (24 hr. EC50)
° U.S. EPA Office of Toxic Substances - Guidelines for Testing of Chemicals: Daphnia Acute Toxicity Test	S or CF	immobility (24 hr. EC50) (48 hr. EC50)
° University of Massachusetts - Acute Toxicity Testing - General Description, Materials and Methods Manual for *Daphnia*	S	lethality (48 hr. LC50)
° The Netherlands - Method for the Determination of the Acute Toxicity of Substances to the Water Flea *Daphnia magna* Straus	S	lethality (48 hr. LC50) immobility (48 hr. EC50)
° British Health and Safety Commission - Approved Test Method for Acute to *Daphnia*	S	immobility (24 hr. EC50) (48 hr. EC50)
° British Dept of Environment: Method for Determination of the 24 hr. or 48 hr. EC50 of Test Substances to *Daphnia magna* *	S, SR or CF	immobility (24 hr. EC50) (48 hr. EC50)

S = static, SR = static replacement, CD = continuous flow

* full test procedure unavailable

Appendix 7A; Part D.

ACUTE LETHALITY TESTING PROCEDURE FOR ARTEMIA

Procedure	**Test Type**	**End-Point**
° Artemia Reference Centre: Standardized Short Term Toxicity Test with Artemia Nauplii (ARC-Test)	static	24 hr. LC_{50}

Appendix 7A; Part E

ACUTE LETHALITY TESTING PROCEDURES FOR MARINE AMPHIPODS

Procedure	Test Species	Test Type	Test Duration	Effects Measured
° E.V.S. Consultants: Laboratory Sediment Amphipod Bioassay Procedure	*Rhepoxynius abronius*	static	10 days	survival and emergence from sediment

Appendix 7A; Part F.

SUBLETHAL ATP ENERGY STRESS TESTING PROCEDURE FOR SALMO GAIRDNERI

Procedure	Test Duration	End-Point
° Environment Canada, Quebec Region: Procedure for Determination of Energy Stress Induced in Rainbow Trout	96 hr.	Minimal Stress Concentration (MSC)

Appendix 7A; Part G.

SUBLETHAL TESTING PROCEDURES FOR PIMEPHALES PROMELAS

Procedure	Test Type	Test Duration	Effects Measured	Endpoint
° U.S. EPA: Fathead Minnow Larval Survival and Growth Test Method	static renewal	7 days	survival and weight changes (growth)	NOEC LOEC ChV
° U.S. EPA: Fathead Minnow Embryo - Larval Survival and Teratogenicity Test Method	static renewal	8 days	percent hatch, percent larval with terata, survival	NOEC LOEC ChV
° ASTM: Standard Guide for* Conducting Early Life-Stage Toxicity Tests With Fish (Draft)				

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* Procedure not available at the time of report preparation

NOEC = No Observable Effect Concentration

LOEC = Lowest Observable Effect Concentration

ChV = Chronic Value

Appendix 7A; Part H.

SUBLETHAL TESTING PROCEDURES FOR DAPHNIA SP./CERIODAPHNIA SP.

Procedure	Test Species	Test Type	Test Duration	Effects Measured	Test Endpoint
° U.S. EPA: *Ceriodaphnia* Survival and Reproduction Test Method	*Ceriodaphnia dubia*	S.R.	7 days	survival reproduction	NOEL LOEL ChV
° ASTM: Proposed New Standard Guide for Conducting Three Brood, Renewal Toxicity Tests With *Ceriodiaphnia dubia*	*Ceriodaphnia dubia*	S.R.	8 days	survival, growth, reproduction	statistical signif. difference
° OECD: Guidelines for Testing of Chemicals - *Daphnia* sp. Reproduction Test	*Daphnia* sp.	S.R., F.T.	14 days	survival, reproduction	EC_{50} LC_{50} NOEL LOEL
° The Netherlands: Chronic Toxicity Protocol for *Daphnia magna*	*Daphnia magna*	S.R.	21 days	survival, growth, reproduction	LC_{50} EC_{50} reprod. coefficient
° ASTM: Proposed New Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*	*Daphnia magna*	S.R.	min. of 21 days	survival, growth, reproduction	statistical signif. difference
° ASTM: Proposed Standard Practice for Conducting *Daphnia magna* Chronic Toxicity Tests in a Flow-Through System	*Daphnia magna*	F.T.	21 days	survival, growth, reproduction	statistical signif. difference

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S.R. = Static Renewal, F.T. = Flow Through
NOEL = No Observable Effect Concentration
LOEL = Lowest Observable Effect Concentration
ChV = Chronic Value

Appendix 7A; Part I.

SUBLETHAL GROWTH INHIBITION STIMULATION TESTING PROCEDURES FOR UNICELLULAR GREEN ALGA

Procedure	Recommended Test Specites	Procedure	Endpoint
° Environment Canada, Quebec Region: Microplate Algal Assay Technique	Selenastrum capricorntum	microplate using cell counts or ATP	4 hr. EC50 96 hr. EC50 8 day EC50
° Environment Quebec: Detailed Method for Quantitative Toxicity Measurements Using the Green Algae Selenastrum capricornutum	Selenastrum capricornutum	flasks using cell counts	8 day IC50
° U.S. EPA Algal Growth Test Method: from Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms	Selenastrum capricornutum	flasks using cell counts, chlorophyll or light asborbance	96 hr. NOEC or 96 hr. LOEC
° U.S. EPA Office of Toxic Substances - Guidelines for Testing of Chemicals: Algal Acute Toxicity Test	Selenastrum capricornutum or Skeletonema costatum	flasks using cell counts	96 hr. EC10 96 hr. EC50 96 hr. EC90
° OECD - Guidelines for Testing of Chemicals: Alga Growth Inhibition Test	Selenastrum capricornutum or Scenedesmus subspicatus or Chlorella vulgaris	flasks using cell counts or light absorbance	EC50 or NOEC (24, 48 or 72 hr.)
° APHA - Standard Methods for the Examination of Water and Wastewaters - 803: Toxicity Testing with Photoplankton, 16th Edition, 1985	no species named	flasks using cell counts	EC50
° ISO - International Organization for Standardization - Method for Determination of Toxicity with Algae	Scenedesmus subspicatus	flasks using cell counts or light absorbance	96 hr. EC50

NOEC = No Observable Effect Concentration
LOEC = Lowest Observable Effect Concentration

Appendix 7A; Part J.

SUBLETHAL TESTING PROCEDURES FOR BACTERIAL LUMINESCENCE INHIBITION

Procedure	Test Species	Comment
° Beckman Microtox^{TM} System Operating Manual	*Photobacterium phosphoreum*	
° Alberta Energy Resource Conservation Board Procedure - Microtox^{TM} Assay Procedure	*Photobacterium phosphoreum*	variation on Beckman procedure
° Alberta Environmental Centre Microtox^{TM} Assay Procedure	*Photobacterium phosphoreum*	variation on Beckman procedure
° Microtox^{TM} Sediment Bioasay Procedure for Testing of Organic Extracts	*Photobacterium phosphoreum*	variation on Beckman procedure
° Microtox^{TM} Bioassay Procedure for Testing of Saline Extracts	*Photobacterium phosphoreum*	variation Beckman procedure

Appendix 7A; Part K.

GENOTOXICITY TESTING PROCEDURES FOR BACTERIA

Procedure	Test Species	Test Duration	Measure	Endpoint
° Maron and Ames: Revised Methods for the Salmonella mutagenicity test (Ames Test)	Salmonella typhimurium	48 hr.	mutagenicity	greater than double the control reversions rate
° Environment Canada, Inland Waters Directorate: Ames Test for Mutagens	Salmonella typhimurium	48 hr.	mutagenicity	greater than double the control reversions rate
° U.S. EPA: Interim Procedures for Conducting the Salmonella/Microsomal Mutagenicity Assay (Ames Test)	Salmonella typhimurium	48 hr. or 72 hr.	mutagenicity	greater than double the control reversions rate
° Israel: Blue SOS Chromotest Kit Instruction Manual	Escherichia coli (K-12)	7 hr.	induction of DNA repair	colourmetric measurement of enzyme induction
° Microbics Corporation: Dark Mutant Genotoxicity Assay*	Escherichia coli	2.5 hr.	mutagenicity	reversion to light metabolism

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* full test procedure unavailable

Appendix 7A; Part L.

BIOCONCENTRATION TESTING PROCEDURES FOR FISH

Procedure	Recommended Test Species	Test Duration	Endpoint
° ASTM - Standard Practice for Conducting Bioconcentration Tests with Fishes (flow-through procedure)	Salmo gairdneri Pimephales promelas Gasterosteus aculeatus	max. 28 days for uptake, max. 28 days for depuration	BCF
° OECD - Bioaccumulation: Sequential Static Fish Test (for use in testing of non-ionized organics)	Ictalurus melas (catfish) Brachydanio rerio (zebrafish)	max. 4 days for uptake, max. 10 days for depuration	BCF
° OECD - Bioaccumulation: Semi-Static Fish Test (for use in testing chemicals of 1 to 500 mg/L water solubility)	Brachydanio rerio (zebrafish)	14 to 18 days, for uptake, (48 hr. test solution renewal) depuration duration not specified	BCF
° OECD - Bioaccumulation: Test for the Degree of Bioconcentration in Fish (for soluble stable chemicals or volatiles)	Orizias latipes (killifish) and Japanese carp	56 days for uptake, no depuration phase	BCF
° OECD - Bioaccumulation: Static Fish Test (for water soluble chemicals)	Brachydanio rerio (zebrafish)	8 days for uptake, no depuration phase	BCF
° OECD - Bioaccumulation: Flow-Through Fish Test (based on ASTM method)	Salmo gairdneri Pimephales promelas Gasterosteus aculeatus	max. 30 days for uptake, max. 60 days for depuration	BCF

BCF = Bioconcentration Factor: the ratio of the test substance concentration in the test fish to the concentration in the test water at steady state.

Appendix 7A; Part M.

BIOAVAILABILITY TEST PROCEDURES FOR MARINE MOLLUSCS

Procedure	Recommended Test Species	Test Duration	Endpoint
° ASTM - Standard Practice for Conducting Bioconcentration Tests With Saltwater Bivalve Molluscs (flow-through, no-sediment)	*Mytilus edulis* (mussel), *Pecten* sp. (scallop), *Crassostrea gigas* (oyster)	max. 28 days for uptake, max. 28 days for depuration	BCF
° Environment Canada, Atlantic Region: Bioaccumulation Testing Procedure for Marine Molluscs (static, sediment added)	*Macoma balthica*, *Mya arenaria*	30 days for uptake, 1 day for depuration	Bioaccumulation

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BCF = Bioconcentration Factor: the ratio of the test substance concentration in the test fish to the concentration in the test water at steady state.

Appendix 7A; Part N.

BIODEGRADATION TESTING PROCEDURES

Procedure	Type Test	Test Duration	Inoculum
° OECD: Modified AFNOR Test	ready	max. of 28 days	effluent from STP
° OECD: Modified Strum Test	ready	max. of 28 days	activated sludge plant effluent from STP
° OECD: Modified MITI Test (I)	ready	14 days	activated sludge mixture from 10 differing locations
° OECD: Closed Bottle Test	ready	28 days	aquecus suspension of garden soil, activated sludge plant effluent, or composite
° Modified OECD Screening Test	ready	max. of 28 days	secondary plant effluent from STP, aqueous suspension from soil, surface water or composite
° OECD: Modified SCAS Test	inherent	max. of 14 days	activated sludge from STP
° OECD: Modified Zahn-Wellens Test	inherent	max. of 28 days	activated sludge from STP
° OECD: Modified MITI Test (II)	inherent	max. of 28 days	activated sludge mixture from 10 differing locations

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STP = sewage treatment plant

Appendix 8

Appendix 8A. A Brief Review of Good Laboratory Practice (GLP).

Appendix 8B. Components of an Ecotoxicological Testing Laboratory QA/QC Program.

APPENDIX 8A

A Brief Review of Good Laboratory Practice (GLP)

GLP has been defined as "...concerned with the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported, and as "...concerned with the quality of test data and the management of laboratory studies" (OECD, 1982). "Furthermore, Good Laboratory Practice, being a management tool, does not interfere with the exercise of scientific knowledge or practice, but rather complements it" (OECD, 1982).

Although GLP embraces virtually every apspect of any measurement process, its major elements would probably be agreed to include the following:

1. All aspects of laboratory operations (such as standard operating procedures (SOPs), project plans, record-keeping, quality control, sources of supplies and organisms, data management, staff training, archives, etc.) are thoroughly documented and this documentation is maintained and used routinely by the laboratory staff.

2. Project management is the responsibility of a single individual. All projects are performed according to written plans, which can only be changed with the consent of the project manager. At the completion of each project, the project manager certifies that the project plan or protocol has been followed in accord with GLP.

3. All projects are monitored for conformity with the overall QA plan by an independent unit or individual. This unit reports separately on its QA audit findings to senior management, and these reports are part of the final project report.

4. External audits of all laboratories are performed by the government agency responsible for the testing by properly trained inspectors in accord with documented audit procedures, in order to ensure that GLP has been followed.

5. Failure to comply with GLP constitutes an offence.

These major elements of GLP have been selected from a large amount of detail in the references cited in Chapter 8 and interested readers will find guidance on its application to virtually all aspects of laboratory operations.

APPENDIX 8B

Components of an Ecotoxicological Testing Laboratory QA/QC Program

The following basic components should be recorded/implemented as a minimum to assuring production of good quality ecotoxicological data:

Test Organisms:	Record species, history, health, acclimation, treatments
Test Material:	Record sampling and storage history, constituents and impurities
Test Methods:	Should be in writing, and consistent with good scientific practice. They should include appropriate control tests and should specify acceptable tolerance limits.
Standard operating Procedures:	Should be written down for easy reference and review on all procedures used in the laboratory
Dilution Water:	Should support normal development of test organisms. Quality should be measured and treatment methods documented
Holding & Test Vessels:	Should be of a suitable non-contaminating material and cleaned thoroughly after use
Instruments:	Should be in good working order and calibrated as necessary
Glassware:	All glassware should be suitably cleaned
Round Robins & Reference Toxicants:	The lab should participate in round robins sponsored by the IGATG and others. In addition, reference toxicant tests should be conducted on each new batch of test fish using as many reference toxicants as practical
Reporting of Results:	Data should be analyzed by recognized techniques. All relevant test details should be reported.
Records:	Records of holding organisms and acclimation; dilution water quality; raw data; sampling and storage history of test materials; and reference toxicant data should be stored for future reference.
Personnel:	Education, training and experience should be recorded. Training should be continually updated.
Safety:	Safety Regulations should be followed (Part IV).

RECOMMENDATIONS ON
AQUATIC BIOLOGICAL TESTS AND PROCEDURES
FOR ENVIRONMENTAL PROTECTION, C & P, DOE

ADDENDUM I

SUMMARY OF QUESTIONNAIRE RESPONSES ON CURRENT AND FUTURE USE FOR AQUATIC BIOLOGICAL TESTING

ADDENDUM I: Summary of Questionnaire Responses* from EP Program Managers on their Current Use/Need/Purpose for Aquatic Toxicity Testing.

T - Technical Services Branch
E - Emergencies and Operational Support Branch
C - Commercial Chemicals Branch
I - Industrial Programs Branch
A - Atlantic Region
Q - Quebec Region
O - Ontario Region
W - Western and Northern Region
P - Pacific Region

* Survey conducted July-August 1986

NOTE: each input comment provided by EP managers is separately identified.

Technical Services Branch

TSB has two major operational arms, the Wastewater Technology Center (WTC) and the River Road Environmental Technology Center (RRETC).

The WTC, located in Burlington, is concerned with the development and assessment of treatment and disposal methods for wastewaters and residues. As such, the primary role of biological tests would be to evaluate toxicity reduction in wastewater treatment systems and to ascertain the acceptability of residue disposal practices.

The current and potential use of biotests in 86/87 projects are identified in T1-T12. It can be seen that the primary needs are for tests to identify and screen toxicity. About 18 of over 90 projects could make use of biotesting, and 4 currently do. While some projects are conducted at WTC, many are at locations across the country. Biological testing requirements must therefore be satisfied at both regional and home-base sites. Presently WTC utilizes W&N and Quebec labs and contract servcies.

The primary clients of WTC are the EP regional offices, provincial agencies and IPB. WTC activities reflect the current concerns of their clients, strategies and standards of EP. Biotest use could be expected to take on a higher priority when elevated to such by their clients.

The RRETC, located in Ottawa, has a responsibility for research and development on oil or chemical spill fate, effects and countermeasures. A multi-disciplinary approach (biology, chemistry, engineering) is utilized.

Biological studies are conducted to support:

- the prediction of impacts for the purpose of contingency planning
- the development and evaluation of spill countermeasures
- spill response decision making on priorities, appropriate countermeasures, cleanup and enforcement.

The current and future use of biotests are generally described in T21-T29. A full range of toxicity testing and biomonitoring techniques are applied. The primary clients of RRETC are other EP branches regional offices. The majority of biotesting is contracted with occasional use of EP regional labs.

TECHNICAL SERVICES BRANCH

I.D.	PROJECT	STATUS
	Wastewater Treatment Center	
T1.	Biological Fluidized Bed Treatment of Complex Energy Producing Industry Wastewaters. Biotesting could be used to assess toxicity removal performance. Need: selective screening battery.	Current Project Potential Use of Biotests
T2.	Fate of Trace Contaminants in Biological Treatment Systems. An estimate of toxicity reduction in municipal wastewater treatment systems is being obtained (input vs. output) to evaluate their capacity to deal with dynamic fluctuations in trace organic contaminants. Using: rainbow trout and Daphnia lethality tests.	Current Project and Use of Biotests
T3.	Full-Scale Evaluation of Anaerobic Treatment Systems. Pilot Scale Assessment of Anaerobic Treatment for Pulp and Paper Wastewaters. Anaerobic Treatment of Complex Industrial Wastewaters. The above pilot scale and full scale demonstrations of anaerobic wastewater treatment systems could use biotests to evaluate toxicity removal efficiency. Using: serum bottle anaerobic assay Need: low volume microtest for pilot-scale Need: screening battery for fullscale	Current Projects and Potential Use of Biotests
T4.	Treatment of EMR/CANMET co-processing wastewaters. This project will evaluate pollution abatement technologies and waste management practices for effluents and residues produced by this technology. Biotesting should be used to determine toxicity removal efficiency on planned bench scale and pilot scale treatment systems. Need: screening battery	Current Project and Potential Use of Biotests

TECHNICAL SERVICES BRANCH

I.D.	PROJECT	STATUS
	Wastewater Treatment Center (Continued)	
T5.	Toxics Identification and Mobility in Sludges. Land Application of Sewage Sludge. Test Methods for Solidified Waste Characterization. Assessment of Solidification Technology NITEP: Solid Waste Management There is a potential for leachate generation in a variety of residue management programs. Biological tests could be of use in screening the leachates to reduce the number of samples. requiring full chemical assessment. Need: low cost, low volume, rapid screening test	Current Project and Potential Biotest Use
T6.	Determine potential for groundwater contamination from the land application of heavy oil wastes. Using: SOS chromotest Algal growth inhibition Microtox	Current Project and Use of Biotests
T7.	Determine if biological degradation can be used to decontaminate soil containing chlorinated organic compounds. Biotesting of soil extracts being conducted. Using: SOS chromotest Microtox Need: other screening tests	Current Project and Use of Biotests
T8.	Demonstrate an activated carbon effluent control technology which concentrates and recycles organics. Biotests could be used to measure toxicity reduction. Need: Screening Test	Current Project Potential Use of Biotests

TECHNICAL SERVICES BRANCH

I.D.	PROJECT	STATUS
	Wastewater Treatment Center (Continued)	
T9.	Full scale demonstration of enhanced effluent clarifiers. Biotesting should be used to assess clarifier performance. Need: rainbow trout or equivalency	Current Project Biotests
T10.	Assess alternate effluent treatment technologies to reduce the toxicity/toxic substances in gold mining and milling operations. Need: screening test battery	Current Project Biotest Use is limited to $s
T11.	Develop, evaluate, demonstrate cost effective technologies for the treatment of waste waters from bitumen/heavy oil operations. Biotesting was used to compare acute toxicity of field samples and will be used to demonstrate organic contaminant removal technologies. Using: Microtox Need: selected acute toxicity tests	Current Project and Use of Biotests
T12.	Demonstrate a mobile pilot scale leachate treatment system. Biotests could be used to assess toxicity reduction. Need: Screening Test	Current Project and Potential Use of Biotests

I.D.	PROJECT	STATUS
	Environmental Emergencies Technology Division	
T21.	Predict threats at an early stage and set priorities for follow-up activities by: (a) screening spill treating agents (for toxicity and bio-availability) at concentrations likely to be encountered during use; (b) screening substances likely to be spilled at concentrations likely to be encountered during a spill (usually high-shock load); (c) screening spilt material (contaminated receiving environment) samples to assist in cleanup/disposal decisions. Use: rainbow trout lethality Need: screening battery	Current and Future Use of Biotests
T22.	Develop and apply prediction models and provide basic toxicity data necessary for their verification. Use: Daphnia lethality and reproduction Artemia lethality	Current Use of Biotests
T23.	Quantitatively assess the toxicity and bio-availability of materials in selected circumstances such as: where indicated by screening (item 21 above); to more fully characterize major spill treating agents; for spill substances (or spilt materials) suspected of causing important effects; those highly toxic; those which bioaccumulate or are persistent. The latter case could involve tests at low-level postspill residue concentrations and chronic exposure conditions. Need: quantitative characterization for toxicity/bioavailability	Historic and Future Use of Biotests
T24.	Experimentally evaluate spill countermeasures (in lab or field) by comparing the biological effects of different treatments and/or no treatment under various environmental and spill conditions. This information to ensure the	Historic and Future Use of Biotests

I.D.	PROJECT	STATUS
	Environmental Emergencies Technology Division (Continued)	
	inclusion of ecological criteria in the selection of the most apropriate option. Use/Need: full range of toxicity and biomonitoring option	
T25.	Determine methods to measure the distribution of materials spilt in the receiving environment i.e.: area of contamination. Need: Indicator/screening test	Potential Project and Use of Biotests
T26.	Document the biological condition of the environment (or indicator components thereof) and components of particular sensitivity in areas where the probability of a spill is high. Need: Baseline monitoring/sensitive bioindicators	Historic and Potential Future Use of Biotest
T27.	Approve or reject chemical dispersant formulations for inclusion on the EPS approved list by testing with the standard protocol in the "Guidelines on the ....Dispersants". Use: rainbow trout lethality Need: equivalency tests	Current Use of Biotests
T28.	Set standards and conditions for use of other spill treating agents and maintain compliance as required. Need: as per T27	Potential Project and Use of Biotests
T29.	Monitor the effects at actual spill sites (and spill cleanup sites) in order to determine the rate of recovery and where applicable evaluate countermeasure effectiveness. This information to be used to refine judgements on correct course of action. Examples of previous activities include Miguasha Marsh, Athol. Use/Need: full range of toxicity and biomonitoring options	Historic and Future Projects and Biotest Use

Emergencies and Operational Support Branch

EOSB, in Ottawa, requires biological testing support in those activities dealing with the Ocean Dumping Control Act. The ODCA stipulates that a waste or other substances may be dumped at sea if it can be demonstrated that the substances are rapidly rendered harmless by chemical, physical or biological processes of the sea.

The current and potential use of biological tests are identified in E1-E6.

EOSB has the primary responsibility for coordination of ODCA activities and development of national standards and protocols. Research is currently being conducted into appropriate biotesting options (Appendix 4A).

Regional offices have the responsibility for implementing the ODCA. Approximately 90% of ocean duping activity involves sea dumping dredged material. Dredging involves OGD's and industry. Current policy requires permit applicants to pay for testing requirements. Inhouse resources are however required for confirmation analysis, dumpsite assessments and compliance monitoring. About 20 permit applications and 14 dumpsite assessments can be expected per year.

Two other divisions reside in EOSB. Currently, however, the Environmental Emergencies Division biotest needs are provided for in regional and TDTSB operations.

Environmental Impact Division does not directly require biotesting but uses results/promotes use in an advisory capacity and as related to the EIA process. One of the major concerns is the need for environmental effects monitoring to evaluate the effectiveness of mitigative measures proposed in the EIS.

EMERGENCIES AND OPERATIONAL SUPPORT BRANCH

I.D.	PROJECT	STATUS
E1.	Develop guidelines to authorize or reject the dumping of substances at sea according to pass/fail biological testing protocol.	Current Program Biotests being evaluated
E2.	Compliance monitoring of above.	Potential Future Use
E3.	Evaluate the effectiveness of permit control measures through dumpsite assessments.	Current Program done jointly with regions
E4.	Determine individual site specific permit conditions.	Current Program done in regions
E5.	Set legal limits for the release of toxic materials.	Current and Future Use
E6.	Apply a tiered assessment scheme to screen/ priorize wastes to be dumped.	Current Need

Commercial Chemicals Branch

CCB, in Ottawa, has a strong interest in the development of testing facilities in the context of their new chemicals program.

They are also assessing the new potential problem of controlling 'biotechnology' waste streams, and the release of bioengineered microorganisms.

Biological test needs to address the latter are unknown. OECD approaches for assessing chemicals are already identified to various degrees of specificity (see Appendix 4B and 4C).

Both programs pose significant implications to biotest use and needs in the future, however, both are contingent on further development and approval processess, e.g., with respect to the Environmental Protection Act.

Suggestions were received that CCB should improve the use of biotests and biological data base required to support contaminant enforcement actions, develop national guidelines and monitor long term trends.

COMMERCIAL CHEMICALS BRANCH

I.D.	PROJECT	STATUS
C1.	Assess the hazard of new chemicals and mixtures using OECD approaches and under conditions of Good Lab Practice. Participate in an internationally coordinated program of chemical evaluation. Initiate biodegradation testing in 86/87 with intent of expanding these and toxicity approaches in future.	Pilot-scale testing for biodegradation to commence at Quebec Lab. $ restrictions on program
C2.	Assess the problem of controlling biotechnology waste streams, and bioengineered microbes.	Current Program; in infancy.

Industrial Programs Branch

IPB, located in Ottawa, has the responsibility on a national scale, for characterization, control and treatment of industrial and municipal wastes, effluents emissions, etc.

Input from IPB is summarized in I1-I24. About half of the candidate projects actually use biotesting and therefore the potential for greater use is substantial. The primary purpose would be in evaluation of waste treatment methods (done in concert with WTC) and the development of national environmental protection standards, codes, etc. These activities require characterization of toxicity and assessing hazards for liquid effluents, residues, sludges, leachates, hazardous wastes emissions, spill substances, etc. The major IPB client is EP itself but also includes OGD's, industry and provincial authorities. To date biological testing has been obtained from the most convenient source at the time.

The application of national standards, enforcement and compliance monitoring is coordinated by IPB, however, implementation is primarily a regional role and therefore, biotesting capability for these activities needs to be located in each region. Those same regions have suggested IPB improve national coordination on evaluating the effectiveness of control measures and strategies.

An important current activity of IPB includes supporting the Ontario government initiative to evaluate municipal and industrial abatement strategies (MISA program). The water quality based strategy includes the evaluation of a number of biotests (see Appendix 4G). Should EP adopt elements of this strategy or that of the U.S. EPA (see Chapter 1.2), then the use of biotests and toxicity in regulating effluents would increase dramatically. Consequently, the required biotesting capability is EP would likewise rise.

A last note is the recent initiative in IPB to develop an assessment-control scheme for hazardous wastes under TDGA. Quality control and compliance monitoring of such a scheme might reasonably be expected to reside in EP labs.

INDUSTRIAL PROGRAMS BRANCH

I.D.	PROJECT	STATUS
I1.	Development of Effluent Regulations for Gold Mines. Requires evaluation of effluent quality and of treatment methods to be non-acutely lethal to fish. Use: rainbow trout legal test Need: ??	Current Program
I2.	Compliance monitoring of metal mines to regulations/guidelines. Use: rainbow trout legal test	Current Use of Biotests (Regional task)
I3.	Assess the extent of groundwater contamination at problem mine sites (operating and abandoned) as part of a national inventory program. Need: screening tests for groundwater	Potential Future Use in Current Program
I4.	Compliance monitoring of regulated discharges from (pulp and paper mills, food processing and meat packing plants. Use: rainbow trout legal test	Current Use of Biotests (Regional task)
I5.	Evaluate the effectiveness of effluent treatment systems, both existing and under development. Need: screening tests/site assessment	Current-ongoing in Conjunction with Regions and WTC
I6.	Reviews and Development of Codes of Good Practice: Could obtain and utilize information from biological testing; e.g. Env. Implications of Forest Products Industry; C.G.P. for Wood Protection Industry; C.G.P. for Wood Preservation Industry; Assessment of Land Disposal of Sludge.	Potential for increased Use of Biotests
I7.	On-site pilot scale evaluation of ultra filtration/reverse osmosis for treating selected fish/fish meal processing wastewaters. Need: screening tests	Potential Use of Biological tests in a Current Project
I8.	NITEP (National Incinerator Testing and Eval. Prog.). Potential use in determining toxicity of ash leachate from full scale energy-from-	Potential Use of Biotests in a Current Program

INDUSTRIAL PROGRAMS BRANCH

I.D.	PROJECT	STATUS
I8.	(Continued) waste facilities and/or comparing to landfill leachate. Need: screening test battery for ash leachate	
I9.	Sewage treatment plant sludge incinerator testing: Compare input and output streams. Need: screening test battery for sludge/ash leachate	Potential Use
I10.	Develop methods and monitor presence and effects of gasoline contaminated groundwater Need: Test to identify contaminant presence and assess effects	Potential Use of Biotests Needs. R&D
I11.	Develop methods and monitor presence and effects of spilled pipeline products. Need: as per I10	Potential Use of Biotests. Needs R&D
I12.	Assess hazard (including humans) of particulate emissions from diesel engines. Test emissions from vehicles using alternate fuel sources and Canadian fuel blends. Need: ames test? screening battery	Potential Program and Use of Biotests.
I13.	Provide advice, monitor the success and failure of MARPOL. Need: Effects monitoring	
I14.	Determine the toxicity of (a) lab leachates from coal fired power plant solid wastes and of (b) wastewaters in order to evaluate site specific mitigative measures for new developments and/or to update the generic code of good practice for steam electric power generation. Used: microtox, algae, trout Need: chromotest? daphnia? screening test/battery	Current Use of Biotests in on-going program

INDUSTRIAL PROGRAMS BRANCH

I.D.	PROJECT	STATUS
I15.	Compliance monitoring of regulated discharges from petroleum refineries. Used: rainbow trout legal test	Current Use of Biotests (Regional task)
I16.	Evaluate biotesting procedures for use in screening and regulating industrial and municipal wastewaters, as part of MISA program. See attachment I(a)	Current Use of Biotests
I17.	Develop a biological screening procedure to characterize waste sludges from natural gas processing facilities. Incorporate these into a decision making process for sludge pond cleanup and site decommissioning. Used: microtox, chromotest, algae, trout, nematode, daphnia Need: a rapid inexpensive screening battery	Current Project. Biotests being evaluated
I18.	Assess methods for the land application of oil based drill cuttings. Biological tests could characterize waste in order to determine application rates. Need: as per I17	Current Project. Biotesting not used because of $ restrictions
I19.	Survey of Paint, Pigment and Ink Industry: Assess hazard, determine acute and chronic toxicity of waste discharges as part of problem assessment exercise. Need: screening battery and quantitative assessment of acute & chronic toxicity	Potential Program awaiting $ approval.
I20.	Remedial Measures for Phosphogypsum Ponds - Technical Review. Biotesting could be used if field testing was required. Need: unknown	Current Project and Potential Use of Biotests
I21.	Treatment Technology Development - Enhanced Metal Recovery from Effluents. Biotests could be used to measure toxicity reduction potential. Need: screening battery	Current Project and Potential Use of Biotests

INDUSTRIAL PROGRAMS BRANCH

I.D.	PROJECT	STATUS
I22.	Environmental Review of the Petro Chemical Industry. Biotesting could be part of the assessment of CPI performance where field sampling programs are planned. Need: screening battery/site assessments	Current Program and Potential Use of Biotests
I23.	Screen and/or assess hazard of landfill leachates in order to set priorities for remedial action. Screen contaminated groundwater samples in order to compare relative toxicity removal and direct chemical analysis. Need: screening test/battery	Potential Use of Biotests
I24.	Development of tiered hazard assessment scheme to be used in a program under Transportation of Dangerous Goods. Solid, liquid and sludge wastes would require characterization in a scheme which would screen for priority regulatory control and reduce/ direct subsequent chemical analysis. Need: tiered ecotox-, hazard assessment scheme	Current Program and Use of Biotests (in infancy)

Atlantic Region

The Atlantic regional office is located in Halifax with a sub-office in St. John's. Both toxicity testing and field biomonitoring procedures are used. Their input is summarized in A1-A29.

The major client is EP Atlantic and secondarily to the Province (e.g. Federal-Provincial Boards) and OGD (e.g. COGLA). Some toxicity testing is done for TDTSB. The Dartmouth and St. John's labs are used to satisfy toxicity testing requirements of the region.

It appears that most testing is directed at either identification and assessment of hazards in various types of samples or at regulatory related programs. Liquid effluents, spills and pesticides problems are emphasized. Also emerging as a prime concern is offshore oil and gas development.

ATLANTIC REGION

I.D.	PROJECT	STATUS
A1.	Develop/recommend water quality objectives or effluent criteria for control of toxicity in waste discharges from offshore production and from offshore exploration. Determine toxicity of effluent discharge streams. Need: to measure acute and chronic lethality, community dynamics, acute and chronic sub-organismic tests.	Potential Use of Biotests. Mechanisms being investigated
A2.	Audit compliance of offshore exploration/production activities to control measures established by joint federal-provincial offshore management boards. Need: as per A1.	Potential Future Activity dependent on nature of control mechanisms established
A3.	Assess hazard of toxics substances and products used in offshore activities, e.g. oil based drilling fluids, anti-fouling paints. Need: as per A1.	Current Use of Biotests
A4.	Determine toxicity of substances likely to be spilled from production of tranportation of petroleum; for use in evaluating potential impact and during spill response (X-ref T2,A4) Use: rainbow trout, Artemia & Daphnia lethality.	Current Use of Biotests. TDTSB program
A5.	Determine the cause/source of non-specific health syndromes or multiple toxic effects in chronically polluted marine environments, e.g. industrialized harbours and inlets. Need for development of tests to address problem.	Potential Use of Biotests
A6.	Legal tests to support prosecutions under the Fisheries Act general provision for spill related events.	Current Use of Biotests
A7.	Determine the potential for impact from new pesticides (prior to registration).	

ATLANTIC REGION

I.D.	PROJECT	STATUS
A8.	Post-Registration Pesticide Evaluation. Verify or complete deficiencies in registration data on effects to non-target organisms or in Canadian conditions. Input to or stimulate re-evaluation process.	Current Program and Use of Biotests
A9.	Determine acceptable site specific application conditions and criteria for pesticide use (input to regional review/advisor committees) The above A7-A9 have made use of: - acute lethality in rainbow trout, stickle back, Daphnia and Mytilis - bioaccumulation in rainbow trout, Mytilis and freshwater clams - lethal & sublethal effects on caged fish or endemic fish - abundance/diversity changes in invertebrates	Current Use of Biotests
A10.	Monitor remedial measures (cleanup) on Sydney tar pond which is a source of PAHs to the marine environment.	Identified as Project for '87
A11.	Screen unknown hazardous wastes (federal) to assess toxicity and guide subsequent characterization and disposal (problem id.).	Current Use of Biotests
A12.	Screen leachates from suspect landfill sites to identify potential toxic problem.	Current Use of Biotests
A13.	Determine adequacy of regulations and guideline, etc. (federal & provincial) in protecting the aquatic ecosystem by environmental effects monitoring. Use: benthic and fish population studies bioaccumulation caged fish studies	Current Use of Biotests - ongoing program
A14.	Quantitatively assess the toxicity and bioavailability of whole effluents within a known problem site in order to determine mitigative actions in-plant (Halifax Airport - federal facility). Use: fish lethality test	Current Project and use of Biotests

ATLANTIC REGION

I.D.	PROJECT	STATUS
A15.	Prosecute violations and establish liability discharges which are out of compliance. Use: fish lethality test	Current Use of Biotests
A16.	Advise/assist industry in toxicity reduction and treatability studies, e.g. determine source/cause or evaluate treatment systems. Use: fish lethality and Daphnia tests	Current use of Biotests
A17.	Monitor/audit the compliance of discharges to toxicity as a controlled and prescribed parameter in the regulations and guidelines of the Fisheries Act or surrogate OGD/ provincial legislation. Use: fish lethality test	Current Use of Biotests
A18.	Determine acute toxicity of chemicals spilled; for use in setting cleanup priorities, legal actions assessing potential damage. Use: fish lethality test	Current Use of Biotests
A19.	Determine the toxicity of crude and refined oils likely to be spilled (X-ref A4, T2). Use: Artemia lethality, Daphnia lethality and life cycle.	Current Use of Biotests. TDTSB Program.
A20.	Determine short term acute toxicity of chemicals frequently spilled or likely to be spilled in Canada (X-ref. T1). Use: rainbow trout acute lethality.	Current Use of Biotests. TDTSB Program.
A21.	Test toxicity of chemical dispersants (preset protocol) to determine if they meet criteria for use in Canada (X-ref. T27). Use: rainbow trout acute lethality.	Current Use of Biotests TDTSB Program
A22.	Determine accuracy/precision of measurements between labs, between methods within samples (QA/QC). Use: rainbow trout lethality and Daphnia life cycle.	Current Program

ATLANTIC REGION

I.D.	PROJECT	STATUS
A23.	Development and evaluation of new biotest methods/protocols (and modifications of existing ones).	Current Activity
A24.	Qualify and quantify differences between lab results and what actually occurs in receiving environment. Determine influence of site specific water quality on responses and tolerances.	Current Use of Biotests
A25.	Assess hazard of sewage from St. John's in order to define treatment needs and/or discharge site.	Potential Project
A26.	Characterize effluent toxicity and bioavailability and assess actual receiving water impact from pulp and paper plant on Exploits River. Demonstrate in effectiveness of existing control measures for purpose of convincing company and province to improve control.	Potential Project
A27.	Rectify problem of quality control of toxicity compliance data generated by Hope Brook gold mine.	Current Use of Biotests
A28.	Assess potential hazard of Stephenville pulp	Potential Project
A29.	Identify potential for impact to water quality from jet exhaust fallout in Labrador.	Potential Project

Quebec Region

The Quebec regional office (Montreal) primarily uses biological tests in the context of tiered ecotoxicological assessment schemes which are developed to be accommodate their problems. Current applications are summarized in Q1-Q10. Pollution controls are primarily affected by the Province. EP takes a lead role on federally implicated operations and on the St. Lawrence River.

Clients include EP Quebec region (primary) and secondarily the Province, OGD's and EP-HQ. Level of effort is a function of the resources provided. Actual biotesting is done using Quebec lab staff and/or using contract support for routine tests with Quebec lab/staff for QA/QC. There is no field biomonitoring capability in house.

Considerable effort in the Quebec lab is also directed to toxicity test development and evaluation, particularly as related to micro tests.

QUEBEC REGION

I.D.	PROJECT	STATUS
Q1.	Assess potential hazard of leached groundwater from federally implicated dump sites (problem id.). Use: Tests used in attachment Q(b).	Current Use of Biotests
Q2.	Quickly assess potential hazard on the ecosystem, including man, from spills of unknown or federally implicated source (problem id.), (spill response). Use: Tests used in attachment Q(a).	Current Use of Biotests
Q3.	Monitor/audit application by DPW of a tiered ecotox approach to assess potential hazard of dredging activity on water column organisms and use. Use: Tests used in attachment Q(b) and Q(g).	Potential Future Activity dependent on DPW acceptance and mode of application

QUEBEC

I.D.	PROJECT	STATUS
Q4.	Assess hazard of new (and existing) commercial chemicals. Follow OECD approach to determine biodegradability. Use: Tests used in attachment Q(c).	Current Use of Biotests
Q5.	Identify potential health hazard to field and lab workers handling unknown samples and contaminated material. Use: Use of direct chromotest.	Current Use of Biotests
Q6.	Assess and priorize different types of industrial effluent discharges in order to establish an adequate action plan for control in a cost effective manner (in concert with HQ/province). Use: Test used in attachment Q(b) and Q(f).	Current Use of Biotests
Q7.	Identify in-plant sources of pollution and/or estimate waste water treatment efficiency (toxicity reduction) in order to establish BPT (in concert with IPB, TDTSB). Use: Test used in attachment Q(e).	Current Use of Biotests
Q8.	Audit Fisheries Act regulations application. Use: Tests used in attachment Q(d).	Current Use of Biotests
Q9.	Provide evidence/specify the deleterious effect according to the general provisions of the Fisheries Act in order to persuade the company and province to act accordingly. Use: Tests used in attachment Q(d).	Current Use of Biotests
Q10.	Evaluate the effectiveness of the Fisheries Act Liquid Effluent Regulations by monitoring and assessing the toxicity of those discharges (post regulation evaluation). Use: Tests used in attchment Q(d).	Current Use of Biotests

KEY TO ATTACHMENTS Q(a) to Q(g)

1	denotes	lethality test available
sl	denotes	sublethality test available
AS/CS	denotes	chronic test available
*	denotes	research ongoing regarding genotoxicity
——	denotes	research and or development needed
**	denotes	tests applicable to marine environment
ATP-stress	denotes	Adenosine triphosphate Energy Stress
AEC	denotes	Adenylate Energy Charge

ATTACHMENT Q(a)

SPILL EMERGENCY

Salmo	1		
Artemia**	1		
Colpidium		sl (growth)	
Thalassiosira**		sl (growth)	
Selenastrum	1	sl (ATP - stress) sl (growth)	AS/CS bioaccumulation*
Photobacterium		sl (metabolic) sl (ultra sensitive)	AS/CS genotoxicity function of cost reduction
Encherichia			AS/CS genotoxicity
Daphnia from Cyst Culture function of reduction in effort	1	sl (mobility) sl (ATP)	AS/CS bioaccumulation*

ATTACHMENT Q(b)

PROBLEM IDENTIFICATION - TIERED EVALUATION

Salmo	1	sl (ATP - stress) sl (AEC)	AS/CS bioaccumulation* AS/CS genotox
Artemia**	1	sl (ATP - stress)	
Selenastrum	1	sl (ATP - stress) sl (growth) sl (AEC)	AS/CS bioaccumulation* AS/CS genotox
Thalassiosira**	1	sl (growth) sl (ATP - stress)	AS/CS bioaccumulation*
Colpidium		sl (growth) sl (AES)	AS/CS bioaccumulation*
Photobacterium		sl (metabolic) sl (ultra sensitive)	AS/CS genotox function of cost reduction function of reduction in effort
Salmonella (AMES)			AS/CS mutagenicity
Escherichia			AS/CS genotox
Pleurodales (TRITON)			AS/CS genotox
Mixed Bacterial Culture		sl (ATP - stress) sl (AEC)	AS/CS Biodegradation (Persistance)
Daphnia from Cyst Culture function of reduction in effort	1	sl (mobility) sl reproduction	AS/CS bioaccumulation*

ATTACHMENT Q(c)

ECOTOX EVALUATION OF PURE SUBSTANCES

Salmo	1	sl (ATP - stress)	Genotox (yolk sac fry) Genotox (tissue culture-hepathocytes
Daphnia from Cyst Culture function of reduction in effort	1	sl (mobility) sl (reproduction) sl (ATP - stress)	AS/CS bioaccumulation*
Selenastrum	1	sl (growth) sl (ATP - stress)	AS/CS bioaccumulation* genotox
Colpidium		sl (growth)	AS/CS bioaccumulation*
Photobacterium		sl (metabolic)	AS/CS genotox function of cost reduction function of reduction in effort
		sl (ultra sensitive	
Pleurodeles (TRITON)			AS/CS genotox
Escherichia			AS/CS genotox
Salmonella (AMES)			AS/CS mutagenicity
Mixed Bacterial Culture			AS/CS biodegradation (Persistence)

ATTACHMENT Q(d)

FISHERIES ACT

Legal Test

Salmo	1		

General Provision

Salmo	1	sl (ATP - stress)	
Selenastrum	1	sl (growth) sl (ATP - stress)	AS/CS bioaccumulation*
Photobacterium		sl (metabolic)	
Artemia**	1	sl (ATP - stress)	
Daphnia from Cyst Culture function of reduction in effort	1	sl (reproduction)	AS/CS bioaccumulation*

Effluent monitoring

Salmo	1	sl (ATP - stress)	
Selenastrum	1	sl (growth) (ATP - stress)	AS/CS bioaccumulation*
Photobacterium		sl (metobolic)	
Escherichia			AS/CS genotoxicity
Daphnia from Cyst Culture function of reduction in effort	1	sl (mobility) sl (reproduction)	AS/CS bioaccumulation

ATTACHMENT Q(e)

PROBLEM IDENTIFICATION & PROBLEM SOLVING

In plant study

Daphnia from Cyst Culture function of reduction in effort	1	sl mobility	
Colpidium		sl (growth)	
Selenastrum		sl (growth)	
Photobacterium		sl (metabolic	AS/CS genotox function of cost reduction function of reduction of effort
Escherichia			AS/CS genotox

Waste water treatment evaluation

Daphnia from Cyst Culture function of reduction of effort	1	sl mobility	bioaccumulation*
Salmo	1	sl (ATP - stress) sl (AEC)	genotox
Colpidium		sl (growth)	AS/CS bioaccumulation*
Selenastrum		sl (growth) sl (AEC)	AS/CS bioaccumulation* AS/CS genotox
Photobacterium		sl (metabolic)	AS/CS genotox function of cost reduction function of reduction of effort
Escherichia			AS/CS genotox

ATTACHMENT Q(f)

GROUND AND SURFACE WATER MONITORING

Photobacterium		sl (ultra sensitive	AS/CS genotox function of cost reduction function of reduction of effort
Daphnia from Cyst Culture function of reduction of effort		sl (reproduction) sl (ATP - stress	AS/CS bioaccumulation*
Selenastrum	1	sl (growth) sl (ATP - stress)	AS/CS bioaccumulation*
Escherichia			AS/CS genotox
Salmo		sl (ATP - stress	bioaccumulation*

ATTACHMENT Q(g)

Niveau Trophique	Organisme	Nature de l'agression	Niveau écotoxicol.	Répose toxique	Temps de réponse	Coût**	Volume D'élutriat par échant.	Personne/ jour par test	No. du test
Bactéries	Photobacterium Phosphoreum (Microtox)	Sous-létale	2	Inhibition de luminescence	5-15 min.	40$	5 ml	0.15	1
	Escherichia Coli (Chromotest)	chronique	3	Potentiel de génotoxicité	2h	170$	5 ml	0.10	2
Algues	Chlamydomonas variabilis	Bioaccumulation	3	facteur de bioaccumulation	1 jour	100$	2 L	0.50	3
	Selenastrum capricomutum*	sous-létale	2	inhibition de croissance	4 jour	30$	5 ml	0.20	4
		sous-létale	2	potentiel de fertitité (eutrophisation)					5
Crustacés	Daphnia* Magna	sous-létale	2	immobilisation	1 jour	150$	0.5 ml	1.00	6
		sous-létale	3	reproduction et mobilité	15-20 jour	350$	1.0 L	3.00	7
Poissons	Salmo* Gairdneri	létale aigue	1	mortalité exploratoire	96 h	50$	20.0 L	0.40	8

* Standard de l'OCDE
** Coût par échantillon, basé sur 10 échantillons

Ontario Region

Input from the Ontario region (Toronto) is summarized in 01-09.

Currently the emphasis on biotest use is related to the characterization and hazard assessment of Great Lakes sediments. Biological tests and procedures were recently evaluated for applicability (see Appendix 4L) to control dredging and disposal of sediments and strategies for managing in place pollutants. It is reasonable to assume that EP Ontario will be called upon in the future to monitor compliance and effectiveness of any control measures imposed since these are boundary waters.

Other potential uses of biotesting in the region are related to post registration monitoring of pesticides and testing of Niagra River groundwater.

Since the Province of Ontario (MOE) is both strong and progressive in controling effluents, there is lesser need for EP Ontario to conduct biotests in the application enforcement or monitoring of national standards.

Government lab facilities available to EP include these in Rexdale (MOE) and Burlington (DFO/C&P).

ONTARIO REGION

I.D.	PROJECT	STATUS
01.	Evaluate, select and standaradize procedures for the measurement of toxicity and the bioaccumulation potential of freshwater sediments. Incorporate these into a tiered hazard assessment scheme and federal-provincial criteria for dredging and disposal of sediments in the Great Lakes (being developed).	Current Program Biotests now besing evaluated
02.	Monitor/audit application of a federal-provincial-state process and hazard assessment scheme for dreding and disposal of sediments in the Grat Lakes.	Potential Future Acitivity dependent on nature of control mechanisms now being established
03.	Determine the nature and impact or potential impact of contaminated freshwater sediments (in-place pollutants) in specific areas with the aim of developing strategies for their management.	Current Activity Biotests are presently being evaluated for selection of those most appropriate
04.	Evaluate the effectiveness of Remedial Action Plans (RAPs).	Potential Future Use of Biotests. Abatement Strategies now beiing written.
05.	Assess hazard from landfill leachates and groundwater near the Niagara River.	Potential Use of Biotests
06.	Set criteria/rule-of-thumb safe levels for the discharge of non-regulated chemicals/compounds.	Suggested national level activity
07.	Establish limits/criteria/scientifically substantiated "action levels" to support ECA enforcement.	Suggested national level activity
08.	Verify and supplement data used in pesticide registration on effects, particularly on non-target organisms.	Potential Project and Use of Biotests
09.	Establish long-term spacial or temporal trends of levels (or effects) of ECA contaminants (by using bioindicator organisms), for problem id. or evaluating effectiveness of controls.	Potential Use of Biotests

Western and Northern Region

Western and Northern regional office is located in Edmonton with district offices in Yellowknife, Regina and Winnipeg.

Their input is summarized in W1-W17. Both toxicity testing and field biomonitoring procedures are utilized.

The major client of the regional labs is EP-W&N (about 77%) and secondarily to EP-HQ (17%) and OGDs (6%). Field biomonitoring procedures are implemented through contracts and in house staff.

Activities emerging as prime concerns in the W&N are those associated with agriculture, energy development, northern development and toxic chemicals management. Emphasis is being placed on regulatory related programs in the area of methods development, and on QA/QC for work conducted in the private sector.

WESTERN & NORTHERN REGION

I.D.	PROJECT	STATUS
W1.	Verify pesticide registration data on prediction of field effects in Canadian conditions. Post-registration monitoring Complete deficiencies in registration packages. Both of above for input to re-evaluation of registration package or to stimulate same. Use: Ames, algal, microtox, Daphnia Need: chronic fish and invertebrate tests.	Current Use of Biotests
W2.	Assess receiving water quality, identify significant sources of toxic effluents and set priorities for action, e.g. North Saskatchewan River. To efficiently direct effort in delineating a problem. Need: screening and chronic tests.	Current Project and potential use of Biotests
W3.	Legal samples of spill events for prosecution under the general provision. Use: rainbow trout legal test.	Current Use
W4.	Determine compliance with federal regulations and guidelines. Use: legal trout test.	Current Use
W5.	Determine effects of 'regulated' effluents on local species. Need: Tests on walleye, pike or perch.	Potential Future Use of Biotests
W6.	Assess hazard of molybdenum in uranium mine effluents (data base deficient). Use: acute toxicity to fish, algae, Daphnia.	Current Use of Biotests
W7.	Evaluate a potential wastewater treatment technology for abbatoir wastes.	Current Activity
W8.	Determine extent of impacted area from spills, e.g. pesticide spill at Lethbridge, e.g. chemical spill on an old bunker C spill. Use: microtox, Bauer respiration toxicology.	Current Use of Biotests
W9.	Development of a screening protocol to determine or predict receiving environment impact (Alberta District Office).	Current Activity

WESTERN & NORTHERN REGION

I.D.	PROJECT	STATUS
W10.	Assess potential effects of chronic contaminant input into Tuktoyaktuk Harbour on indigenous benthic fauna (benthic biological monitoring). Use: contaminant body burdens, histopathological analysis and MFO induction in a fish, clam, and amphipod.	Current Use of Biotsts
W11.	Evaluate the effectiveness of existing site specific control measures on the Norman Wells oilfield. Use: indigenous fish tissue and bile analysis for contaminants and fish liver histopathology	Current Use of Biotests
W12.	Assess toxicity of process chemicals, e.g. sodium bromide/BioD as a substitute for cyanide in gold mining. Need: acute and chronic tests.	Current Use of Biotests
W13.	Advise regulatory authorities on impacts and control requirements for offshore oil and gas activities (toxicity testing and/or field biomonitoring) in Beaufort Sea, e.g. on toxicity of oil based drill cuttings.	Current Activity
W14.	Evaluate wastewater treatment systems to advise Territorial Water Board on license conditions.	Current Activity
W15.	Assess methods for measuring effects to marine benthic fauna to determine those most suitable for marine monitoring programs.	Current Program
W16.	Assess freshwater monitoring techniques.	Current Activity
W17.	Monitor non-regulated activities, point sources in the NWT to screen for problems.	Current

Pacific Region

The Pacific regional office is located in Vancouver with a district office in Whitehorse. Their input is summarized in P1-P36. Both toxicity testing and field biomonitoirng procedures are used.

A strong emphasis is placed on the need to show in-situ or 'real' effects. The use of indigenous and/or resource species in toxicity testing is likewise considered important. Site specific control measures are commonly developed and applied. A wide range of clients are served and actual toxicity testing is conducted by the EP lab in Vancouver or with the support of the DFO lab.

PACIFIC REGION

I.D.	PROJECT	STATUS
P1.	Develop environmental controls for the regulation of offshore drilling wastes. Baseline sediment and tissue metal data are collected. Proposed assays on drilling muds using amphipods, flatafish, cod, clams, etc., chromosome aberration tests and in-situ recolonization for long term effects.	Current and Future Use of Biotests
P2.	Assess damage to fisheries resources from contaminants in Vancouver Harbour. Determine effects and contaminant patterns to focus in on sources/priorize control actions. On-going are measurments of contaminants in biota, gross tissue abnormalities in fish and invertebrates, histopathological evaluations. Sediment and effluent bioassays on marine species are proposed.	Current Use of Biotests
P3.	Evaluate site specific control measures at B.C. coastal mines. On-going are measurements of bioaccumulation in resource species and lower trophic levels. Proposed are invertebrate sediment bioassays, metallothionein assays and infaunal recolonization studies.	Current Use of Biotests
P4.	Determine toxic contaminant distribution patterns in the Fraser estuary and Georgia Strait. Proposed are contaminant uptake studies, invert.-sediment bioassays, anaphase aberration tests, monitoring for tissue disorders.	Current Use of Biotests
P5.	Assess potential hazard and impacts of the contamination in the Fraser estuary.	Proposed Use of Biotests
P6.	Evaluate effectiveness of site specific control in protecting resources adjacent to coastal pulpmills.	Current Use of Biotests

PACIFIC REGION

I.D.	PROJECT	STATUS
P6.	(Continued) Completed are benthic population and salmon abundance surveys. Ongoing are sediment-tissue analysis. Proposed are in-situ salmon fry and herring toxicity, and egg bioassay.	
P7.	Assess environmental effects of marine sewage discharges. Determine level of contamination and sewage toxicity. Completed are sediment-tissue analysis, toxicity to herring larvae. Invert.-sediment assays are proposed.	Current Use of Biotests
P8.	Assess environmental impact of industrial spills to marine waters. Determine level of contamination and potential hazard of the substance.	Current Use of Biotests
P8a.	Determine sublethal and chronic effects of pentachlorophenol on resource species.	Proposed Project and Biotest
P9.	Evaluate on a national basis the effectiveness of control measures now in place.	Suggested national level activity
P10.	Assess whether control measures are protecting the environment on a site specific basis.	
P11.	Develop an in-situ/field system to monitor and assess the biotic response of freshwater stream biota to site specific effluent controls (either proposed or inplace). The approach would be recommended to the company/province. Response monitored: Heavy metal accumulation, metallothinein induction, biomass accrual chrolophyll-a) Response (future needs): avoidance, sediment bioassays (fish embryo/larval), various physiological/histological techniques, growth, alkaline phosphate; coldwater benthic invertebrate culture for stream assessments.	Current Project

PACIFIC REGION

I.D.	PROJECT	STATUS
P12.	Assess an in-situ fish cage test for monitoring pulp and paper discharges. Response monitored: Acute lethality or acute sublethal response (leucorit). Response (future needs): Chlorinated organics accumulation (in-situ exposures), physiological testing to assess fish condition iln conjunction with long-term exposure.	Current Project
P13.	Determine the toxicity of the effluent from a pilot treatment system for a TMP/CTMP pulpmill and evaluate receiving water quality. Response monitored: acute lethal and acute sublethal responses being considered (96-h LH_{50}) 24-h stress bioassays (leucocrit, bloodsugar and chloride, temperature challenge, hypoxia challenge, salinity challenge, UDP-glucuronyl transfer-ase enzyme activity).	Current Project
P14.	Advise province and company on monitoring program adequacy and requirements for Quinsam Coal development. Baseline data collection and assessment of methodology for freshwater monitoring programs. Response monitored: Standing crop (chlorophyll-a), biomass/density, taxonomy Response (future needs): nutrient limitation (onsite evaluation) and various physiological and compositional prarameters to determine nutrient sufficiency.	Current Project
P15.	Assess chronic toxicity of coal dust suppressent chemicals leached from coal storage piles.	Potential Project
P15a	Assess chronic toxicity ethylene dichloride associated with ground spills.	Potential Project
P16	Assess toxicity of various air contaminants and industrial emissions (thrust in USA).	Suggested national program

PACIFIC REGION

I.D.	PROJECT	STATUS
P17.	Determine long term toxicity of industrial effluents.	Potential Project
P18.	Assess potential hazard of a potential spill (contingency planning/impact of prediction), e.g. toxic chemicals transport on Thompson River.	Current Project joint with TDTSB
	Use: acute toxicity.	
P19.	Monitor for the presence of a spill substance to determine if countermeasures or cleanup should continue.	Current Activity
	Use: short-term rainbow trout test.	
	Prosecute spill violations under the F.A.	
P20.	Use: legal trout test.	Current
P21.	Indicate the compliance of regulated effluents. Need a rainbow trout test equivalent, a cheaper and quick screen.	Current Use
	Use: rainbow trout legal test, microtox and Daphnia.	
P22.	Determine acute toxicity of pesticides to local species to advise control agencies.	Current Use of Biotest
	Use: juvenile Pacific salmon.	
P23.	Monitor post application pesticide residues.	Potential Use of Biotests
P24.	Improve acceptability/legal ease for using information other than 96 hour fish acute lethality in court, i.e. substitute test with smaller animal, i.e. sublethal and chronic effects.	General Comment on Need
P25.	Apply rapid in-situ tests to direct activities in spill response/enforcement.	General Comment on Need

PACIFIC REGION

I.D.	PROJECT	STATUS
P26.	Aid courts in 'sentencing' violations under the Fisheries Act by demonstrating/clarifying an effect beyond lethal and establishing linkages with real resources and receiving environment. Use of sublethal and chronic tests to show potential impact.	General Comment on Need
P27.	Need simple and quick screening tests to identify a problem exists for remote office or field case (e.g. Yukon).	General Comment on Need
P28.	Monitor the receiving environment to assess adequacy of control measures. Need standard sublethal biological tests related to environmental quality (Yukon).	Current Use of Biotests and need for improvement
	Presently use benthic invertebrate studies.	
P29.	Legal tests for prosecutions of Fisheries Act either out of compliance with regulations or general provisions. Need simple and cheap equivalency tests for 96 hour trout bioassay (Yukon).	Current Use and future need
P30.	Assess toxicity of mining and municipal effluents or contaminated material with simple/cheap process rather than rainbow trout test.	Current Use of Biotests and future need
P31.	Determine environmental threat of contaminated groundwter seepages (problem identification) in remote locations such as the Yukon.	Potential Use of Biotests
P32.	Develop/select tests which will indicate human health concerns.	General Comment on Need
P33.	Develop/select toxicity tests on indigenous species in order to demonstrate resource impact.	General Comment
P34.	Develop/select biotests indicative of results from community trend analysis.	General Comment of need

PACIFIC REGION

I.D.	PROJECT	STATUS
P35.	Define when an 'effect' is significant. Determine 'level of protection' for a variety of needs.	General comment of need
P36.	Strengthen relationships between toxicity tests and real in-situ effects and between test species and indigenous species.	Comment of need